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ON THE PHOTOGENIC ORGAN OF THE KNIGHT-FISH (*MONOCENTRIS JAPONICUS* (HOUTTUYN)).

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It was Stead (1906, p. 89) who first described the luminescence of the knight-fish (*Monocentris gloria-maris*). "On each side of the head near the mouth are peculiar luminous discs, which are probably of service to the fish in assisting it to obtain its food." Unfortunately this interesting observation has escaped attention up to this date.

Monocentris japonicus, another well-known species of the knight-fish, is a rather common animal along the coast of Japan. This fish also has the same property of light production as the Australian form. Light is produced by a pair of glands situated just under the lower jaw of the fish. Their function and structure were described by Yoshizawa (1916, p. 411) in Dobutsu-Gaku-Zashi, a Japanese zoölogical periodical. His brief description was illustrated with figures, but several points remain unexplained. According to Yoshizawa the photogenic organs of *Monocentris* have the construction of photospheres, composed of large glandular cells with nuclei of a considerable size, but without accessory structures such as the reflector. He seems not to have been aware of the truly glandular nature of the organs.

In 1917 Harvey came to Japan and made at the Misaki Biological Station some observations upon the interesting luminous shore-fish. These observations have been recorded in several of his papers. The photogenic property of *Monocentris* thus has come to be generally known as a fact.

Localization and General Structure of the Photogenic Organs.

The photogenic organs of *Monocentris* consist of two ovoid protuberances lying side by side on each side of the median thickening of the lower jaw, their longer diameter being nearly at right angles to the median plane (Fig. 1, *gl*). In a fish 12 cm. long the protuberances measure about 4 mm. in length and 3 mm. in width. Their whole surface being covered with minute dermal papillæ of dark brown color, the photogenic protuberances do not stand out clearly above the surrounding

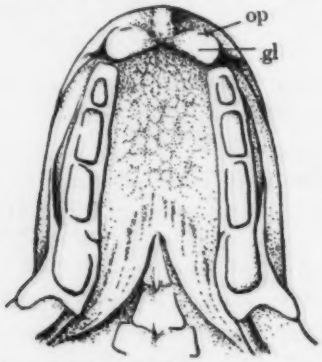

Fig. 1. Ventral view of the lower jaw of *Monocentris japonicus*. About × 2. *gl*, photogenic gland; *op*, opening of gland.

surfaces. This fact seems to be the reason why the organs in question have remained unnoticed by most ichthyologists.

The photogenic organs of *Monocentris* are glands of an excretory nature, although luminous material does not escape under normal conditions. The glandular nature of the organs can easily be demonstrated even without dissection. Each photogenic protuberance has a slit-like opening at the anterior end (Fig. 1, *op*) and when pressed under conditions of darkness a luminous fluid is observed to be forced out through this point. The opening is crescentic and its longer diameter is almost parallel to that of the protuberance.

The interior of the photogenic organs is white and spongy, containing a large quantity of minute crystals. A great number of simple tubules develop around the central tissue.

They crowd so thickly that each stands almost vertically, and they lie parallel to one another. Indeed, the tubules appear to belong to a single gland with several large central spaces (Fig. 3, pl. I.).

Each photogenic organ of *Monocentris* is, however, a compound gland made up of some nine components which unite secondarily into a functional whole. The distal part of the gland still shows the compound nature and this part consists of many narrow canals, each representing the duct of an individual acinous

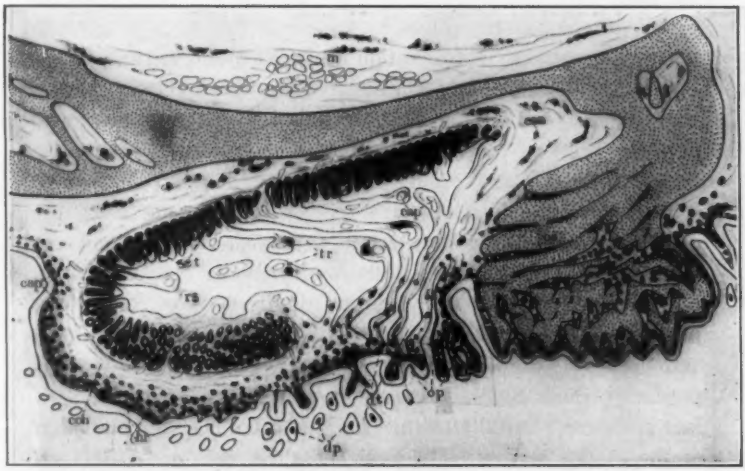

FIG. 2. Sub-mental photogenic gland of *Monocentris japonicus* in longitudinal section passing through its opening. About × 30. *cap*, capillaries; *chr*, chromatophores; *con*, connective tissue; *dp*, dermal papillæ; *dt*, emissory ducts; *m*, muscles; *ri*, reservoir; *op*, openings of gland; *os*, sub-maxillary bone; *se. t*, secretory tubules; *tr*, trabeculæ.

gland. The general constitution of the organ is represented in Fig. 2.

HISTOLOGY OF THE PHOTOGENIC ORGANS.

Under a high magnification each tubule shows an epithelial lining composed of cubical cells arranged in a single layer except at the fundus region which is the seat of a most active secretion, where the walls are considerably thickened with two or three cell layers (see Fig. 4, pl. I.). The stratified aspect of this

region is, however, not due to the direction of cutting but is the result of actual proliferation of epithelial cells. Toward the exterior at a certain distance from the fundus the stratified aspect is no more to be noticed, the cells being generally lower and the secretion less in amount. Thus, in transverse sections of the tubule, the fundus and distal region show a structural difference, the walls of the former being thick and stratified, while those of the latter are thin and simple. Naturally the difference is a gradual one. The caliber and length of the tubules are also variable, but the largest ones are generally situated at the proximal region of the gland.

A certain quantity of secretion is always found in the tubules. It is granular in composition and readily stainable with hæmatoxylin. The epithelium of the tubule is completely destroyed in the process of secretion instead of repeating its function a number of times as ordinary secretory epithelium does. Each cell of the epithelium, in this case, does not function independently but operates in connection with others along the considerable extent of the walls of the tubule. The new epithelium is replaced by the constant proliferation of cells. The process is especially active at the fundus region of the tubule, and here the process is so active that more than one layer is produced before the uppermost one is entirely destroyed. This fact gives rise naturally to the stratified aspect of the epithelium at the fundus region. The nuclei of these cells are ovoid and contain the chromatic substance in normal amount. They do not deteriorate in a degree visible to the eye as far as the cellular destruction occurs. By the time when the cell body is completely disintegrated and the nuclei are set free into the lumen of the tubule, the latter lose their internal structure, their chromatic substance being reduced in amount. Yet the nuclear membrane remains unaltered and shows, adhering to its inner surface, the last scattered traces of the chromatic substance (Fig. 5, *n'*, pl. I.).

As has been stated, the secretion is of granular constitution. Whether or not the granules are preëxistent in the cells is difficult to say. They have been described in the cells of *Anomalops* and *Photoblephalon*, two luminous shallow-water fishes in the

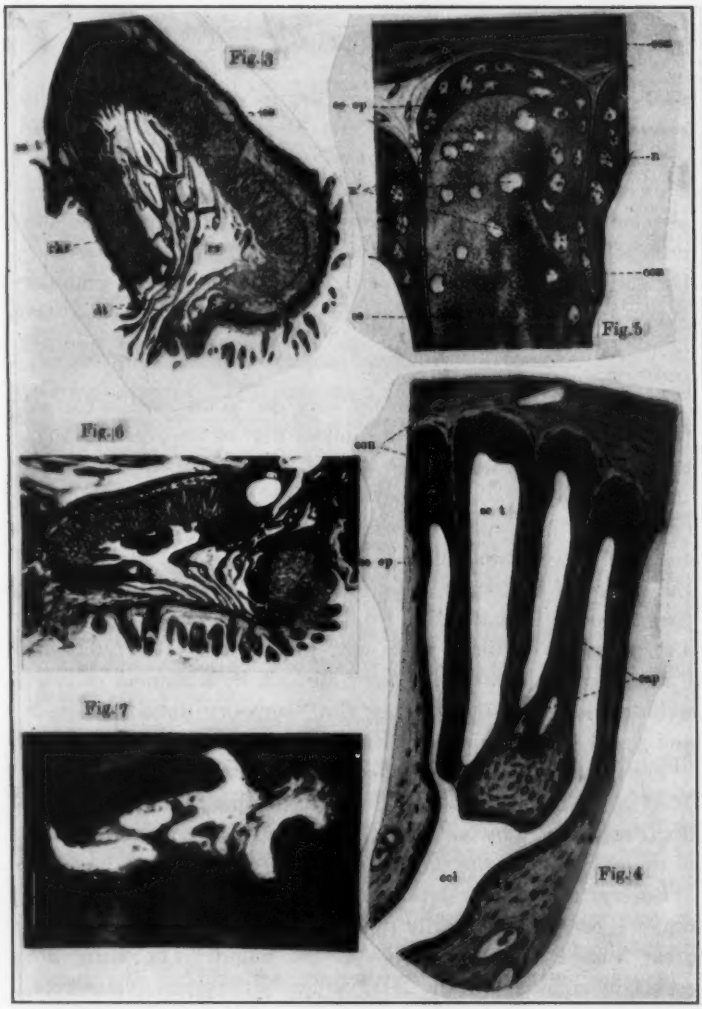

FIG. 3. Sub-mental photogenic gland of *Monocentris japonicus* in horizontal section at the level of its opening. × 16. *chr*, pigment layer; *dt*, emissory ducts; *rs*, reservoir; *se*, secretion; *se t*, secretory tubules.

FIG. 4. A portion of gland proper. × 640. *cap*, capillaries; *col*, collecting tubule; *con*, connective tissue; *se ep*, secretory epithelium; *se t*, secretory tubule.

FIG. 5. Basal part of a secretory tubule. × 1,250. *con*, connective tissue; *n*, nuclei in epithelium; *n'*, nuclei in secretion; *se*, secretion; *se. ep*, secretory epithelium.

FIG. 6. Sub-mental photogenic gland of *Monocentris japonicus* viewed by transmitted light. × 16.

FIG. 7. The same gland seen by reflected light. Crystalline deposit is represented in white.

Malayan Sea, and the photogenic organs of these fishes closely resemble those of *Monocentris* (see Steche, 1909, p. 349). No granules have, however, been detected in the cells of the latter. I am rather of the opinion that the granules are produced through the metamorphosis of the cytoplasm at the time of cellular destruction as Förster (1914) has described in *Pholas dactylus*. On the other hand, Harvey (1921, p. 43) claims the granular bodies in the tubules to be symbiotic bacteria which cause the luminescence. To this problem I shall return on another occasion.

The secretion produced in the way just considered is carried from the tubules toward the medullary part of the organ through a number of tubules of the second order, or the "collecting tubules" (Fig. 4, *col*). The latter proceed more or less parallel to one another but separated by irregular trabeculæ of connective tissue. They communicate often in their course and finally at the centre of the organ constitute several large lumina, or the "reservoirs" (Figs. 2 and 3, *rs*), in which the secretion is stored and probably oxydized with light production. The reservoirs communicate also with the outer medium by a number of long, well-defined, parallel tubes, or the "emissory ducts" (Figs. 2 and 3, *dt*). About nine such ducts are generally observed.

The inner walls of the collecting tubules and reservoirs are lined by a low, simple epithelium, while those of the emissory ducts are covered by one which is stratified, a direct continuation of the epidermis of the surface.

Beyond the tubular area—the gland proper—the photogenic organ is enclosed externally by a thick dermal layer in which a great number of chromatophores are found. The latter are especially abundant on the outer surface of the organ. However, they are scattered and do not constitute a pigment cap (Figs. 2 and 3, *chr*).

The blood is supplied by branches of the lower jaw arteries which run near the organ. They enter into the gland proper, passing through the surrounding dermal tissue, and on reaching the tubular area branch out all at once into many capillaries, each making its way between the secretory tubules. After passing the tubular region the capillaries gather themselves again

to form veins which enter into the connective-tissue trabeculæ of the medullary part of the organ.

The tissue of the central part, and to some extent that which encloses the proximal part of the organ, is always loaded with a large quantity of very minute crystals which appear chalky white by reflected light and brown by transmitted light (comp. Figs. 6 and 7, pl. I.).

Harvey states that the luminescence of *Monocentris* is continuous. The fish can produce light both day and night. But the emission of light seems to be controlled to some extent by internal stimuli. The operating mechanism has not, however, been completely ascertained. No special muscle fibers have been found in the organ. Nevertheless it may not be unjustifiable to suppose that the thin stratum of more or less elastic fibers surrounding the outer surface of the tubular region exerts a contraction by which the spontaneous light is produced. Otherwise it is quite difficult to understand the spontaneous luminescence occasionally produced by the fish. This stratum is not clearly differentiated from the ordinary dermal tissue.

I have not been able to detect any nerves entering the gland proper.

Observations on the Living Fish.

In connection with this morphological study on the photogenic organs of *Monocentris* I have made some biological observations on the living fish. The emission of light was seen in darkness under certain conditions. It could easily be produced by agitating the water in which the fish was kept or by adding to it a few drops of ammonia, but very often the luminescence was spontaneous. Luminescence is continuous for even several hours. By rubbing the luminous protuberances with a piece of stick or by scraping them with a knife edge, the luminosity could partially be transferred to the surface of the stick or knife, remaining visible there for several seconds. Although it will be seen that in this case we are dealing with a substance excreted by the organs, it should not be considered that the luminescence is ordinarily due to excreted matter—the "external luminescence"—because no luminous material was seen excreted into the water by a living fish; the luminescence is "extracellular" but "intraglandular."

Monocentris can live in fresh water for a considerable length of time, even for ten hours. Nevertheless, they are quite uneasy under such conditions, as indicated by rapid beats of the fins, stirring movement of the tail, a bubble formation on the skin and, especially, the emission of a continuous bright light.

Monocentris can live in the sea water within a range of temperature between 0° C. and 40° C., and light is produced within somewhat wider limits, the maximum temperature being about 42° C. and the minimum about − 10° C.

Monocentris can show light at any time of the day and night, but the natural luminescence seems to cease in the day time, for the fish after being removed from daylight to darkness did not show this property at least for ten to fifteen seconds.

The regeneration experiment of the photogenic organs, which I have performed, may be interesting and is somewhat suggestive in regard to the problem of the animal luminescence in general. The photogenic organs of eight fishes were excised in varying extent. The wound healed very soon and in the course of about two weeks the integument was completely regenerated. The internal changes following these operations were studied by means of serial sections. The result shows that the tubules of the organs, either completely or partially removed, are not regenerated but are replaced by a spongy tissue consisting of exceedingly enlarged blood vessels. If the granules in the organ were bacterial bodies, they might have been expected to increase in amount, because they were subjected to a favorable condition with an abundant supply of blood. But this does not seem to have been the case. There was no increase in the amount of granular matter in the remaining tubules.

Similar Photogenic Organs of Other Fishes.

Finally the only photogenic organs which at all approach those which have been considered are to be found in two Malayan fishes, *Anomalops katoptron* (Bleeker) and *Photoblepharon palpebratus* (Boddaert) which have been made the subject of memoirs by Steche (1907, p. 85 and 1909, p. 349). A glance at his figures in Pls. XIX–XXI will at once show the fundamental similarity of

the "sub-ocular" organs of the *Anomalopidæ* and the "submental" organs of the *Monocentridæ*. However, the reservoirs and emissory ducts which are well developed in *Monocentris* are wanting in both *Anomalopsis* and *Photoblephalon*. In the latter all of the several secretory tubules communicate with the external medium by one very short common duct. But each photogenic organ in question is an aggregation of many acinous glands developed in a limited area sub-ocular in position, and the secretory tubules stand vertically and lie parallel side by side as in the case of *Monocentris*.

The crystalline deposit is also present. The chromatophores are described as forming a pigment cap which encircles the proximal surface of the organ.

In *Anomalopsis* the oral end of the photogenic organ is said to be fastened to a long cartilagous shaft, upon which it is rotated downwards so as to bring the luminous surface against the body wall and thus cut off the light. In *Photoblephalon* the action of shutting off the light is done by a moveable screen. No analogous mechanisms of light regulation have been found in the submental photogenic organ of *Monocentris*.

LITERATURE CONSULTED.

Forster, J.
'14 Zeitschr. f. wiss. Zoöl., 109, 349–.
Harvey, E. N.
'17 Carnegie Inst., Washington, Pub., 251, 223–.
'19 The Nature of Animal Light, 69.
'21 Science, N.S., 53, 314–.
'22 Carnegie Inst., Washington, Pub., 312, 43.
Stead, D. G.
'06 Fishes of Australia, 89.
Steche, O.
'07 Verh. d. deutsch. zoöl. Geselsch., 17, 85–.
'09 Zeitschr. f. wiss. Zoöl., 93, 349–.
Yoshizawa, S.
'16 Dobutu-Gaku-Zashi, 28, 411.

THE DEPENDENCE OF SECONDARY SEX-CHARACTERS UPON TESTICULAR HORMONES IN LEBISTES RETICULATUS.

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I. INTRODUCTION.

The problem of sex as a chapter in the mechanics of development has not been investigated until lately. A number of authors whose investigations were performed on different animals have proven that those sex-characters which are generally called secondary sex-characters depend in their development, on hormones produced in the sex glands. After castration these characters will disappear. Proof to this effect has been produced by Steinach, Sand and Lipschütz in mammals; Pezard, Goodale and Zawadawsky in birds; Harms, Meisenhaimer, Witschi, Bresca and others in amphibians.

The dependence of secondary sex-characters in fishes was not at all investigated until quite recently. The reason for this may be found in the fact that those species which are endowed with permanent dimorphism, the exotic inhabitants of the aquaria, are distinguished by a very scant size and want of stability and therefore, are almost inaccessible for operative intervention.

As to seasonal dimorphism we find in the works of Courrier (2) and Von Oordt (5, 6, and 7) on the stickleback (*Gasterosteus pungitius* and *G. aculeatus*) indirect indications of its dependence on the hormones of the testis.

A direct experiment was carried out by St. Kopeć (4) who succeeded in castrating *Phoxinus lævis* males and females and keeping them alive for three weeks after operation. During this time said fish failed to exhibit nuptial colors at the usual time.

The hybridological analysis of the sex-characters in fishes was carried out by Aida (1) on *Haplochihis latipes* and by Winge (8 and 9) on *Lebistes reticulatus*. Both authors came to the conclusion that the color characters of the male depend on the genes located in the sex-chromosome, especially in the Y-chromosome.

The *Lebistes* female, even if its X-chromosome contains genes of color characters, does not phenotypically exhibit the latter.

II. Description of Material and Methods.

In connection with genetic researches on *Lebistis reticulatus* I had the opportunity to observe a great many of those little fishes in the Moscow aquaria. Among the members of this species as well as among members of other species which have come under my observation I very seldom met with divergencies from usual dimorphisms.

My material consists of seven fishes, six of which are males with marked divergence from typical dimorphism. The seventh is a case of hermaphroditismus glandularis. The malformation of dimorphisms of the males were either inborn or developed later in the life of the individuals. Two males, No. 25 and No. 34, belong to the former class.

1. *Male Fish No. 25.*—The individual measured 37 mm. in length. It was entirely deprived of dimorphic colorations. The shape of the body resembled that of a female. Upon dissection, after the fish died, it was discovered that the testis was entirely atrophied.

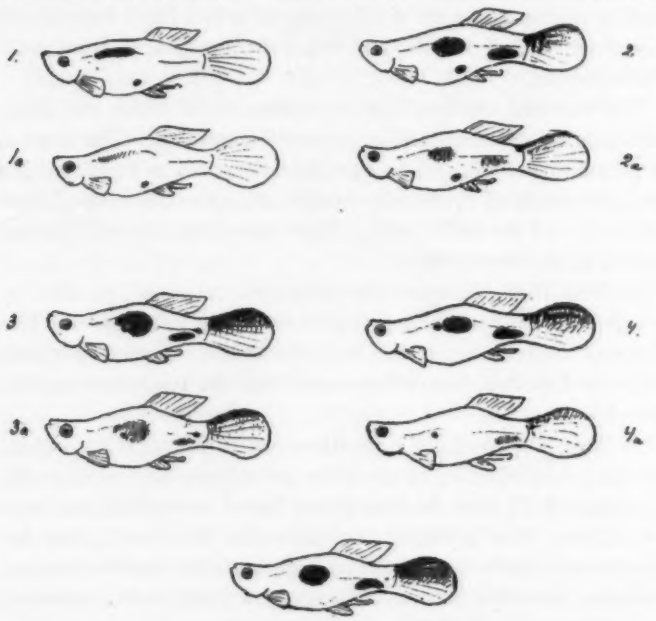
2. *Male Fish No. 34.*—It was born on the 5th of August, 1924. On the 5th of May, 1925, when the animal was nine months old, it measured 23 mm. in length but failed to exhibit any sex-colorations. The gonopod was normally developed. Sex instincts were not lacking; repeated mating to fecundate females, however, proved a failure. No sign of a gonad was discovered macroscopically.

3. *Male Fish No. 23.*—This animal reached maturity in the first part of September, 1924. Up to February of the following year it remained quite normal as to dimorphic colors and sex-instincts. During February the characteristic red spot on the side of the body disappeared and the fish became almost colorless. It died on the 27th of May, 1925, and on dissection revealed entirely atrophied gonads (Figs. 1 and 1*a*).

4. *Male Fish No. 8.*—With normal dimorphism this animal reached maturity in August, 1924. Paleness of sex-colors was noticed in March, 1925. On April of the same year both of the

red spots were almost invisible. Also the black spots found on the side of the body lost their intensity. The specimen died on May 24, 1925. A very slightly developed testis was found after examination of the viscera. (Figs. 2 and 2*a*.)

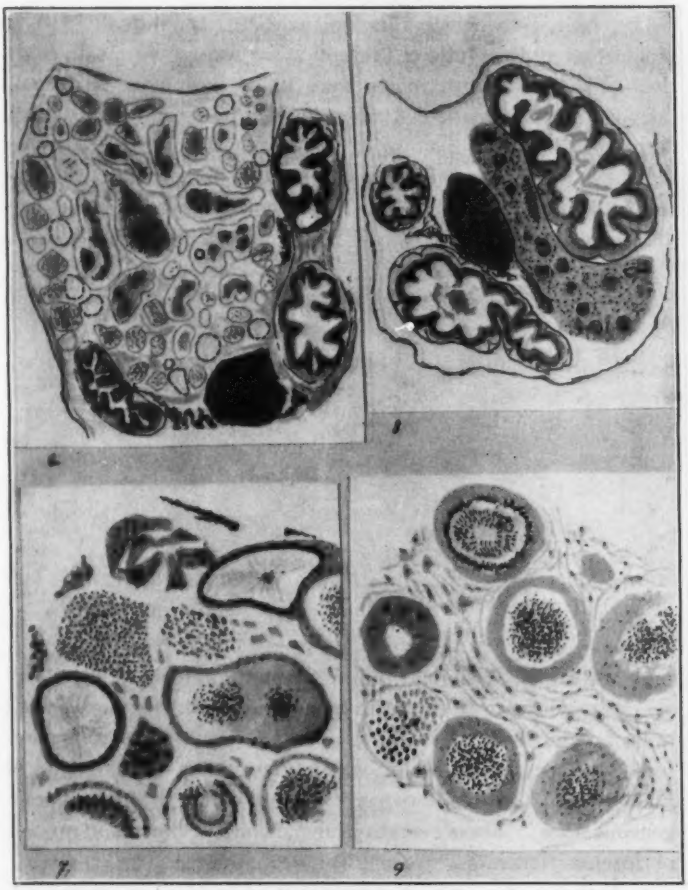
5. *Male Fish No. 33.*—The specimen was born in August, 1924, and reached maturity with secondary sex-characters well formed. From May, 1925, on, the characteristic male shape was under-



FIGS. 1–5.

going a change toward that of the female. This was closely followed by the disappearance of sex colorations. Figs. 3 and 3*a*. On June 4, 1925, it was killed and preserved in formol-alcohol solution. Sections eight microns thick were cut and stained in hematoxylin. Upon examination it was found that the testis was very small and almost deprived of spermatocysts. It contained neither primordial germ cells nor spermatocytes. Spermatogenesis was encountered here and there but only the later stages. The connective tissue of the gonad was well developed.

The coincidence of these two facts—loss of dimorphic colors and the atrophy of the testis—appeared significant and stimulated me to a more detailed study of the last "pale" male No. 44,

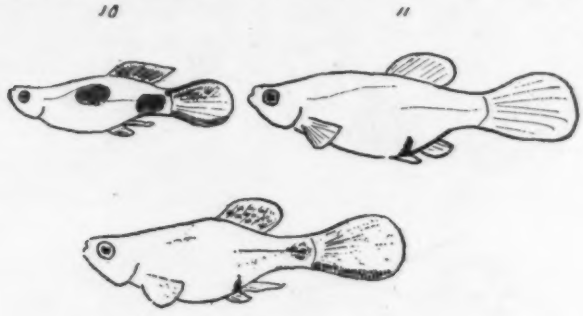


FIGS. 6–9.

Figs. 4 and 4*a*, parallel with its quite normal brother No. 47, Fig. 5.

6. *Male Fish No. 44.*—Phenotypically both fishes, No. 44 and No. 47 were very much alike. Both had two red spots in each

side with the upper part of the tail fin colored yellowish-orange. Male No. 44 had three black spots on each side while No. 47 possessed only one on each side. They were born on the 18th of September, 1924. Both fish developed and remained alike until May, 1925, from which time on male No. 44 gradually lost its coloration and on June of the same year was, so to speak, colorless (Fig. 4*a*). Both males were killed and fixed in formol-alcohol, sections made 6–8 micron in thickness and stained with hematoxylin. The histological picture of the normal male No. 47 showed a large testis and not less than 70 spermatocysts can be counted on one section. The connective tissue is poorly developed (Figs. 6 and 7). In case of male No. 44, the testes is



FIGS. 10–12.

only one fourth as large as that of its normal brother, only 16–17 spermatocysts can be found in one section with spermatogenesis almost reduced to nil. The connective tissue however is very well developed (Figs. 8 and 9). In the middle of the gonad of male No. 44 are found two cysts filled with an almost homogeneous mass. These cysts appear to be much like ova in process of degeneration.

7. *Fish No. 59. The Hemaphrodite*—For this specimen I am very much indebted to Mr. N. J. Dragomiroff, who sent it to me from the University of Kiew. This fish had died 6 months of age and reached me preserved in Tellyesniczky's fluid.

The body of the *Libistes* hermaphrodite measures 37 mm. in length, which is a female characteristic, for the males never exceed

25 mm. The dorsal fin is rounded as is the case in normal females Near the anal orifice a dark black spot can be seen which characterizes the mature female in the viviparous fishes and which has been called "puberty spot" by Essenberg (3). The anal fin however has been modified towards the male direction and resembles a gonopod of not fully developed males, (Fig. 12). The colorations are more like those of the male than those of a female. The black spot found in the tail region is present in the hermaphrodite as well as the orange line on the lower margin of the tail fin. Compare Fig. 10, which is a normal female, Fig. 11,

FIG. 13.

a normal male, and Fig. 12, the hermaphrodite. On dissection a fully developed ovary containing almost completely formed embryos was discovered.

The gonad was embedded and sections, 8 microns in thickness, made. Material was stained in Heidenhain's iron-hematoxylin and Mallory's connective tissue stain.

The histological picture corroborates all the features that were observed by the unaided eye, that is, a well-developed ovary with embryos almost ready to be born. Besides all along the ovary, gradually tapering, was found a well developed testicle (Fig. 13).

Contiguous with the testicular tissue lies a mass containing disintegrated ovarian tissue (compare with Essenberg, 1923, Table 5, Fig. 25). In the testicular portion the various stages of spermatogenesis can be found. To all appearance normal and mature sperm cells gathered in cysts (Spermatophores) are just as abundant and regular as can be observed in normal testis.

Discussion and Summary.

It was observed in the description of the six *Lebistes* males that the atrophy of the testis is paralleled by the disappearance of the male sex colors. Based upon these facts the writer concludes that the intensiveness, shape and development of the black and especially the red and yellow pigment spots depend upon the hormones produced in the testis in Lebistes.

With the disappearance of the male sex coloration the individual approaches the colorations of the female, that is to say, the female coloration is the characteristic coloration of the asexual forms.

In the case of mammals, birds and amphibians it is known that the coloration of the asexual forms approaches the sex colorations of that sex which is homozygos as to sex chromosome composition. In *Lebistes*, therefore, the male is heterozygos (XY) as to sex chromosome composition and the female is homozygos (XX).

Winge (8 and 9) has found in *Lebistes reticulatus* the presence of the gene complex S which is located in the X-chromosome and which is transmitted sex-linked. In other species of *Lebistes* (comprising the majority of the species of this genus), the X-chromosome is empty, that is devoid of color factors, and all color characters are transmitted by the Y-chromosome (one sided). The phenotypical manifestation of the S-complex in the males described above consists in the yellowish tinge of the dorsal fin and the orange line along the lower border of the tail fin and possibly the contour of the red spot on the tail.

No doubt the genes of the color pattern of the hermaphrodite belong to the S-complex. These are the orange line along the lower edge of the tail fin, and the red pigment on the tail Any other color characters the genes of which are located in the Y-chromosome, could not be found. Winge states that the Y-

chromosome never is devoid of color factors. If that be so, our hermaphrodite lacks the Y-chromosome and from a genetic point of view it is a female.

LITERATURE CITED.

1. **Aida, T.**
'21 On the Inheritance of Color in a Fresh-water Fish, *Aplocheilus latipes*. Genetics, 6.

2. **Courrer, R.**
'22 Etudes preliminaire du determinisme des caracteres sexuels secondaires chex les poissons. Arch. d' anat. d' histol. et d' embriol., T. 2.

3. **Essenberg, J. M.**
'23 Sex-differentiation in the Viviparous Teleost, *Xiphophorus helleri*. BIOL. BULL., Vol. 45, No. 1.

4. **Kopeć, S.**
'18 Contribution to the Study of the Nuptial Color of Fishes. (Polish with english summary.) Sprawozdania Taw. Nauk, Warsz. Rok., XI.

5. **van Oordt, G.**
'23 Secondary Sex-characters and Testis of the Ten-spined Stickleback. Proc. kon. Akad. v. Wetensch, Amsterdam, v. 26.

6. **van Oordt, G.**
'24 The Relation of the Testis to the Secondary Sex-characters of the Ten-spined Stickleback. Tijdschr. d. Ned. Dierk. Vereen. Dl. 19. Aft. 1.

7. **van Oordt, G.**
'24 Die Veranderung des Hodens wahrned des Auftretens des Sekundaren Geschleshtamerkmale bei Fischen. *Gasterosteus pungitius*. Arch. f. Mikr. Anat. u. Entw.-Mech., 40, 102.

8. **Winge, O.**
'22 One-sided and Sex-linked Inheritance in *Lebistes reticulatus*. Journ. of Genetics, Vol. 12, No. 2.

9. **Winge, O.**
'23 Crossing over between X- and Y-chromosomes in *Lebistes*. Journ. of Genetics, Vol. 13, No. 2.

10. **Zawadowsky, M.**
'22 Das Geschlecht und die Entwicklung der Geschlechtsmerkmale. Moscow, Staatsverlag. (Russian with German summary.)

11. **Zawadowsky, M.**
'23 Die Entwicklungsmechanik des Geschlechts, Moscow, Russia, Staatsverlag

THE RÔLE OF THE NUCLEUS IN THE CELL FUNCTIONS OF AMŒBÆ.

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The importance of the cell nucleus in chemical synthesis of cell elements, regeneration, cell division, fertilization, and heredity is now appreciated. There are, however, many problems connected with rôle of the nucleus in the ordinary, everyday life of the cell which have not been so fully worked out. To many of these problems the metazoan cell forbids an approach because it is too differentiated, too specialized. In addition, it is difficult to eliminate the factor of interrelationship of cells in making such studies on the rôle of the nucleus. For this and other reasons the protozoa have supplied favorite objects for such studies. Because they lead a more or less autonomous existence they exhibit their vitality in a greater diversity of activities than do metazoan cells.

The researches of Gruber, Balbiani, Verworn, Morgan, Hofer, Stolć and many others have supplied ample evidence that an enucleated protozoan is incapable of regenerating any lost cell structure; *e.g.*, Morgan found that as much as 1/64 of a protozoan, *Stentor*, will produce a new individual if it contains a nucleus, but not if one is absent. The non-nucleated part will heal the wound, but will not regenerate lost cell organelles. Verworn too found that if he bisected a Foraminiferan, *Polystomella*, the part without the nucleus would not regenerate the material for a new shell as the nucleated portion did. An apparent exception is the contractile vacuole of amœbæ which, it is generally agreed, always appears anew in an enucleated fragment of an amœba not possessing one at the time of merotomy.

In ciliates generally locomotion is not affected by removal of the nucleus. There are, however, certain factors bearing upon the locomotion, irritability and nutrition of the cell, especially in the amœbæ, concerning which there is much disagreement. The method generally used in studying the bearing of the nucleus

upon any phase of cell activity is by removing it, and observing any variations from the normal in the behavior of the cell.

A. Gruber (1886) was perhaps the first to cut an amœba into two pieces for the purpose of observing the effect of the removal of the nucleus upon locomotion. He says (free translation), "The part with the nucleus continues to project and withdraw its pseudopods, as before but the part without a nucleus withdraws its pseudopods although there is a weak streaming at first. Two days after the bisection the (enucleated) amœba died." He concluded that the experiment showed that in amœbæ the capacity for movement is affected, which he had found was not true in the case of the Infusoria. Hofer (1890) found in a large number of experiments that the removal of the nucleus exerted a direct influence upon the movement of the protoplasm, and he therefore concluded that the nucleus is a regulatory centrum for the locomotion of the amœba. He also found that enucleated amœbæ ceased to form the secretion wherewith amœbæ attach themselves to the substratum. His descriptions and figures show that at no time did the fragments free from nuclear influence approach normal locomotion. Willis (1916) observed a number of enucleated amœbæ for seventy-two hours. He observed that during this time their movements were jerky, irregular and very much slower than those of nucleated parts. They were able to attach themselves to the substratum only weakly and for short intervals of time. Lynch (1919) found that the enucleated part, seven minutes after amputation, ceased its progressive movements and retracted into a corrugated sphere. After one or two days some of the nucleated fragments commenced to move characteristically, but were not able to attach themselves to the slide. Against all this evidence, we have the work of Stolć (1910) who reaches the rather amazing verdict that enucleated amœbæ show the same characteristic movement as nucleated ones, including the feature of attaching themselves to the substratum. Furthermore, he showed to his own satisfaction that enucleated amœbæ were as irritable as nucleated ones, and exhibited the same stimulated condition.

It is evident that it is impossible to gain from the literature any adequate conception of the bearing of the nucleus upon the

locomotion of amœbæ. It was for this reason that the writer undertook the experiments reported in this paper. The amœba employed throughout was *Amœba dubia* which is characterized by an ovoid nucleus, lack of ectoplasmic ridges, and the possession of many pseudopods, with no axial pseudopod. Thanks are due to Dr. J. A. Dawson for giving assistance in way of amœbæ and technique in starting the cultures. After the cultures were once well started, little difficulty was experienced in carrying them on.

From their figures, it appears that Hofer used the type of amœba with the ovoid nucleus, possibly *A. dubia*, while Stolć used an amœba with a biconcave nucleus, probably *A. proteus* or *A. discoides* (See Schaeffer, 1916). The others employed "*A. proteus*," but from their figures it is impossible to determine which species of the *proteus* types.

The instrument employed in my experiments in removing the nucleus was a fine, hard glass point drawn out in a micro-burner, by means of which the portion of the amœba containing the nucleus was severed from the remaining portion. In all cases the enucleated portion was slightly larger than the nucleated. Each portion of the amœba was then placed in a watch glass in a large drop of fluid from a culture in which amœbæ were actively growing, and kept in a moist chamber for observation. The water was changed twice daily so as to be kept as fresh as possible.

Records were kept of sixty-four enucleation experiments, although many more were performed with similar results. The length of life of the enucleated fragments was somewhat variable, as follows:

Two amœbæ lived only one day; eleven, two days; eighteen, three days; sixteen, four days; eleven, five days; four, six days; one, seven days; and one lived eight days. Thus the greater number lived between three and four days. This may be compared with Hofer's enucleated amœbæ which lived from eight to twelve days (one more than fifteen days) and those of Lynch which lived for from six to eight days. Stolć claims to have been successful in keeping amœbæ artificially enucleated alive for as much as twenty-five days. Strangely enough, however, two enucleated fragments which arose from two different amœbæ that had divided spontaneously lived only ten and four days

respectively (Stolć, 1910). It is possible that some of the variations in length of life are due to the species of amœbæ employed by the various workers.

In order to ascertain the effect of the removal of the nucleus upon the locomotion of the amœbæ, sixty-four experiments were performed, and carefully recorded. Each amœba was under observation for a few hours after cutting, and was studied at frequent intervals afterwards so long as it showed any activity. The following experiment is typical of the greater number of the experiments and will be given somewhat in detail.

An amœba moving actively in a healthy culture (Fig. 1) in a Syracuse watch glass was bisected at 3:44 so as to stimulate it as little as possible. The protoplasmic streaming was not discontinued in either piece (Figs. 2 and 3). The fragments moved away, however, in opposite directions from the plane of cleavage, as invariably happens when a clean cut is made so as not to agitate the amœba too much. At 3:45 both portions were still moving, attached to the substratum, as determined by directing a fine stream of water from a pipette upon them. At 3:49 both were still progressing normally. But from 3:49 to 3:54 the activity of the enucleated part slowly diminished (Fig. 4), it lost its hold on the substratum, and the extended pseudopods appeared literally to shrink into short, stubby, wart-like processes (Fig. 5). Under the higher power of the microscope it was apparent that the surface of the fragment was wrinkled and that the clear zone of ectoplasm around the periphery had disappeared, the granular protoplasm extending throughout. Meanwhile the nucleated portion continued to move quite naturally. Later it was placed in a moist chamber where it ingested food and divided two days later. From 3:54 to 5:23 the enucleated fragment remained in the irregularly spherical shape, with the surface considerably wrinkled (Fig. 6). The shape changed somewhat from time to time, but almost imperceptibly. At 5:23 it sent out a long pseudopod (Fig. 7) and commenced a slow streaming, but because it was not attached to the substratum there was no progressive movement. At six o'clock it was attached and streaming actively in an irregular limax fashion (Fig. 8). During the next two days it was attached and streaming actively in a

limax fashion when observed (Fig. 9). On the third day the streaming was slower, change of shape was not so rapid, the attachment to the substratum was loosened, and the periphery of the amœba was a wide zone of clear ectoplasm (Fig. 10). On the fourth day perceptible streaming and change of shape had ceased, and the amœba was called dead.

From the series of experiments, certain definite facts were learned. First, locomotion is not at once affected by the removal of the nucleus if the amœba is not agitated in the process of cutting. Second, from five to ten minutes after enucleation the amœba commences to stream more slowly. This is accompanied by a gradual release of its hold on the substratum. Third, the surface of the enucleated portion becomes wrinkled, as if the pressure from within were reduced. Lynch too mentions the fact that his amœbæ assumed the shape of a corrugated sphere about seven minutes after the removal of the nucleus. Hofer's figures show that he too obtained it. It appears as if the removal of the nucleus causes the cell to lose turgidity. Entire amœbæ placed in 20 per cent. sugar solution become wrinkled in a similar manner after about fifteen minutes. Third, in about two and one half to three hours after enucleation the streaming is resumed, the amœba attaches itself to the substratum, and moves in a manner approximating normal streaming. More often, however, it assumes a *limax* shape, and moves after the fashion of a monopodal amœba, sometimes forming lateral pseudopods, but only temporarily. Fig. 16 shows an enucleated amœba moving in an extremely normal fashion two days after merotomy. Concerning the causes why the amœba should again become active after an interval of comparative inactivity, one can only speculate. There must be a certain amount of pressure within an amœba before it can project a pseudopod. Mast says that it is the contraction of the plasmagel and the hypertonicity of the plasmasol which provides the mechanics of locomotion. The pressure within is reduced when the nucleus is removed, but is increased to some extent after an interval of two to three hours. Certainly then the nucleus is not the centrum for controlling the locomotion of the amœba. Rather, due to some unknown physical cause, its removal seems to affect the imbibition of

water into the protoplasm. Later the property of imbibition is reacquired. If the contractile vacuole is in the enucleated fragment at the time of enucleation, it disappears completely when the fragment becomes wrinkled (Figs. 5 and 6) and does not again appear until streaming is resumed (Fig. 7). Fourth, enucleated fragments, often show a tendency to move in a limax or monopodal fashion. Fifth, the removal of the nucleus does not affect noticeably the ability of the amœba to adhere to the substratum and thus approximate normal locomotion. Sixth, the solation-gelation (Mast 1923) process continues in enucleated amœbæ.

It should be noted, however, that in many cases where to casual observation it appears that an enucleated fragment is moving normally, a closer scrutiny and comparison with a normal amœba shows that the movement is not quite natural. It is difficult to get any quantitative data regarding the degree of normalcy of amœboid organisms. The tendency to move in a *limax* fashion, which *A. dubia* rarely does normally, is the most perceptible deviation from the normal.

There are other irregularities which one finds, such as the tendency of the enucleated *limax* form to flow in one direction for a while, and then very suddenly contract at the anterior end and flow in the opposite direction. Occasionally an amœba will flow in opposite directions from the middle for a short time, in which case the neck of protoplasm connecting the two streaming units becomes very thin. These phenomena seldom occur in normal nucleated specimens.

Stolć has made the assertion, and laid much stress upon it, that enucleated amœbæ show the same irritability as nucleated ones. That is to say, they show what he has designated as the equilibrium condition when streaming unmolested (Fig. 1.), and the stimulated condition when stimulated mechanically (Fig. 11). In experiments like the one described above, the amœbæ were not disturbed for a while after cutting. A number of experiments were performed in which the amœbæ were disturbed just after cutting and at other times. The following is a typical experiment.

At 3:12 an amœba was cut into two approximately equal

pieces. They were drawn up into a pipette and transferred to another watch glass. Both went into the typical stellate stimulated condition (Figs. 11 and 12). At 3:20 the nucleated portion (*A*) was still suspended in the water in the typical stimulated condition. The enucleated portion (*B*) had lost all but two of its processes, and the entire surface of the amœba was rough and wrinkled (Fig. 13). At 3:26 (*A*) was still stimulated, but (*B*) had withdrawn into a wrinkled sphere (Fig. 14). At 3:30 (*A*) was about the same, but (*B*) had sent out a pseudopod with a rough surface. At 3:40 (*A*) was attached and flowing normally. (*B*) was still in a wrinkled condition but with a long wrinkled pseudopod (Fig. 15). At 3:50 both were again drawn into a pipette and shot out again in the stream of water. Both went into the stimulated (stellate) condition, but in just an instant (*B*) became again a roughened sphere. (*A*) remained in stimulated condition for the next ten minutes. At 4:45 both were again drawn into the pipette and shot out in a stream of water. (*A*) showed the typical stimulated condition, but (*B*) went at once into a wrinkled sphere. At no time during the three days of life of the enucleated portion (*B*) could it be made to respond to the stimulus in the normal manner, as did (*A*), which responded in the same manner as the normal entire amœba.

Thus it is apparent that at first both portions show the typical stimulated condition, but the enucleated portion loses the normal response withing a short time. It can respond only by forming a wrinkled sphere, as if it were unable to exert sufficient pressure from within to support the pseudopods. If one watches the "arms" of the stellate enucleated fragment after the initial stimulation following soon after the operation of cutting the amœba into two pieces, the arms appear to melt away, the surface being thrown into folds. The writer can offer no better explanation than that the degree of turgidity becomes insufficient to sustain the pseudopods. Thus we see that the reactions of amœbæ to mechanical stimuli are very much affected, the verdict of Stolć to the contrary. The enucleated fragment has not lost its irritability, but it no longer gives the normal response.

The nutritional processes of amœbæ, especially the ingestion and digestion of food have been further points of controversy.

According to Hofer, Lynch and others, enucleated fragments of amœbæ do not ingest food. Stolć declares that amœbæ ingest food in a perfectly normal manner for many days after enucleation. Schaffer (1920) has pointed out that feeding calls for concerted streaming, and whether enucleated amœbæ ingest food or not is certainly of great importance in evaluating the importance of the nucleus in cell regulation of amœbæ.

Figure 17 shows an *A. dubia* in the normal act of feeding with the interesting observation of one smaller food cup superimposed upon one arm of a large food cup. In culture fluids swarming with *Chilomonas* and *Euglenæ*, the writer has never observed an enucleated amœba ingest a food substance by means of a food cup. It has been observed that such a fragment may flow over a *Chilomonas*, when it appears as if a small cup were formed in the under side of the pseudopod, but on no occasion was the act of enclosing the flagellate and engulfing it into the protoplasm completed.

Schaeffer (1922) has mentioned that by stimulating an amœba with a fine glass rod by waving it in the water some distance from the amœba, the amœba may form a food cup (Fig. 27). While this reaction is not difficult to produce in the case of an entire, healthy amœba, in the case of an enucleated amœba there is either no reaction to a vibrating needle, or only an abortive pseudopod is extended (Fig. 28).

Although enucleated amœbæ which had ingested very large *Euglenæ* could often be found, one was never observed in the act of feeding. Once the writer put a large enucleated fragment of an amœba in a drop of water containing many *Euglenæ* and sat down at the microscope to watch it until the process of feeding should be observed. It was noted that *Euglenæ* which had no flagella often approached the amœba by the typical "euglenoid movement," but the amœba did not react positively toward it. After about an hour it was observed that an *Euglena* was battering the amœba in its attempts to go forward (Fig. 18). Instead of showing an "avoiding reaction," it would simply recoil, and make a new attempt to go forward in the same direction. Soon it had literally battered an entrance into the amœba (Figs. 18, 19, 20, 21). Finally the channel was deep enough to enclose

the *Euglena*. The protoplasm of the amœba closed over the entrance, the *Euglena* struggled a bit, then doubled up, and a food vacuole was formed around it (Fig. 22). Such ingested *Euglenæ* are often digested, although sometimes they are egested very soon after entering the amœba. The process of digestion can be followed. First the *Euglena* gradually loses its green color, becoming brown in an hour or so; then it is reduced in size, and finally after many hours only some brown undigested remains are left in the food vacuole. The vacuole shows the acid reaction up to the end of the process, as proved by its intense redness to a very weak neutral red solution in the water.

Thus, while normal feeding reactions have never been observed in enucleated amœbæ, nevertheless, food may be ingested by an amœba, the organism ingested entering the amœba by its own efforts, and not by any positive activity on the part of the amœba. This method of feeding certainly does not call for concerted streaming. The inability of the amœba to form a food cup seems to be due to the lack of pressure from within sufficient to sustain the pseudopods engaged in the circumvalation of the food particle. Food once ingested can be digested by the amœba after the fashion of normal amœbæ.

In five instances not included in the sixty-four experiments, the enucleated fragment of the amœba had divided spontaneously in from twelve to forty-eight hours after merotomy. The writer chanced to see one such fragment stretched out as in Fig. 23. The streaming was essentially like that of two *limax* amœbæ jointed at their posterior ends. The neck joining the two became narrower and narrower, until the edges rounded off (Fig. 24), leaving two fragments moving in a *limax* fashion (Figs. 25 and 26).

While this is not, of course, normal cell division, it is interesting to note that a cell without a nucleus may divide. The division appeared to be due to the pull exerted in this region by the two halves tending to move in opposite directions, and an accompanying increase of surface tension at the point of division.

Conclusions.

Enucleated amœbæ of the species *Amœba dubia* show the following properties:

1. Within a few minutes after enucleation streaming ceases, and the amœba becomes a wrinkled sphere.

2. In a few hours the streaming is resumed.

3. This streaming is usually of the *limax* type, though it may approximate the normal.

4. Enucleated amœbæ may attach themselves to the substratum.

5. Enucleated amœbæ are irritable, but do not show the same response to stimuli which nucleated amœbæ show.

6. Food organisms which enters the body of an enucleated amœba are killed and digested in an apparently normal manner.

7. Enucleated amœbæ may become divided by antagonistic streaming currents within.

BIBLIOGRAPHY.

Gruber, A.

'86 Beitrage zur Kenntniss der Physiologie und Biologie der Protozoen. Berichte der Naturforschenden Gesellschaft zu Freiburg, 1: 1–24.

Hofer, Bruno.

'90 Experimentelle Untersuchungen uber den Einfluss des Kerns auf das Protoplasmas. Jena. Zeit. f. Nat., 17: 105–175.

Lynch, Vernon.

'19 The Function of the Nucleus of the Living Cell. Am. Jour. Phys., 48: 258–283.

Mast, S. O.

'23 Mechanics of Locomotion in Amœba. Proc. Nat. Acad. Sci., 9: 258–261.

Morgan T. H.

'01 Regeneration of Proportionate Structures in Stentor. BIOL. BULL. 2: 311–328.

Schaeffer, A. A.

'20 Amœboid Movement. Princeton Press.

Stolć, A.

'10 Ueber kernlose individuen und kernlose teile von *Amoeba proteus*. Arch. f. Entwick., 29: 152–168.

Willis, H. S.

'16 Influence of the Nucleus on the Behavior of Amœba. BIOL. BULL., 30: 253–270.

DESCRIPTION OF PLATE.

(Free-hand drawings of actual specimens, except where stated otherwise.)

1. *Amœba dubia* in normal locomotion.

2. The fragment containing the nucleus, immediately after merotomy.

3. The fragment without a nucleus, containing the contractile vacuole.

4. Enucleated fragment in which streaming is arrested. The hold on the substratum is lost, and withdrawal of pseudopods and wrinkling of the surface has commenced.

5 and 6. The same fragment in the wrinkled condition. The contractile vacuole is not visible in this condition.

7. Streaming and pseudopod formation resumed, but not yet attached to substratum. Contractile vacuole again appears.

8. Streaming of enucleated fragment with pseudopod formation.

9. Limax streaming with small lateral branching, typical of enucleated fragments.

10. Enucleated fragment, practically dead.

11 and 12. Both fragments in stimulated condition.

13. Transitional stage to 14.

14. Wrinkled sphere appearing soon after typical stimulated condition in enucleated fragments.

15. Projection of pseudopod.

16. An enucleated amœba, moving in an almost normal manner, which has ingested and partially digested a number of Euglenæ.

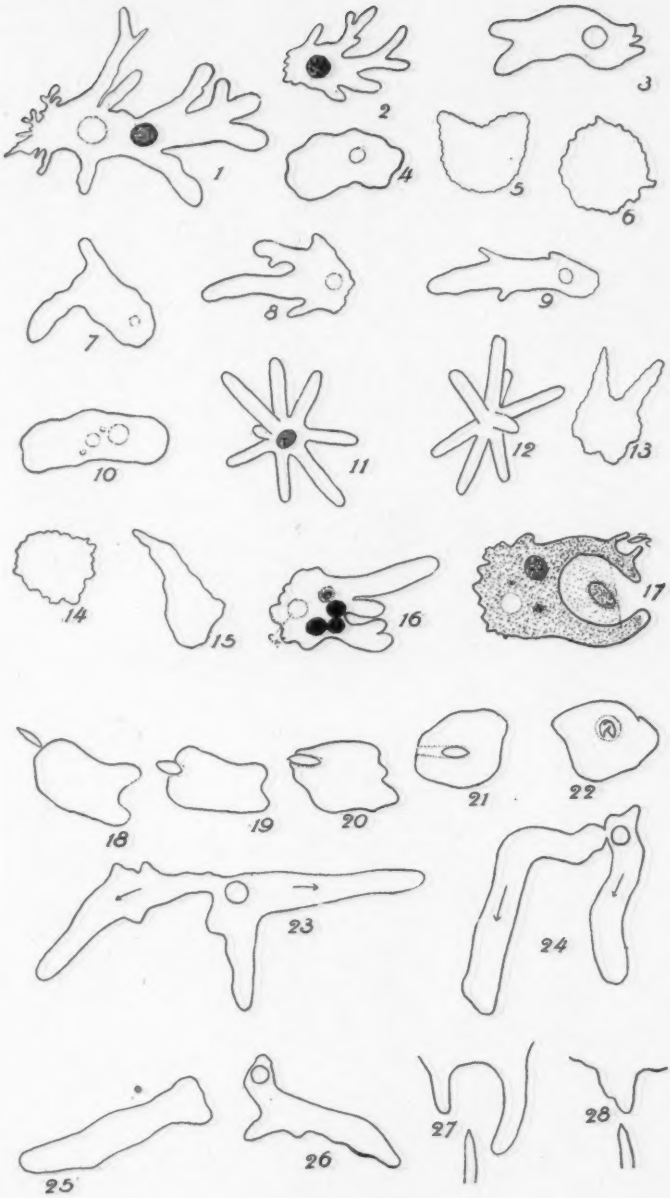
17. Food cup superimposed on a food cup of normal amœba in act of feeding.

18 to 22. An *Euglena* entering an enucleated amœba with final food vacuole formation.

23 to 26. An enucleated amœba dividing autonomously (camera lucida drawings).

27. Food cup induced in an entire amœba by agitating tip of glass rod in water.

28. Abortive reaction of enucleated amœba to the same stimulus.



ELERY R. BECKER.

PALM AND SOLE STUDIES.

IX. The Morphology of the Hypothenar of the Hand; a Study in the Variation and Degeneration of a Typical Pattern.

HARRIS H. WILDER.

Basing our conclusions upon the results of morphological observation, mainly those of Miss Whipple, 1904, it is safe to assume that a friction-skin pattern, whether a "finger-print," *i.e.*, one taken from the apical mound of a finger, or one located upon the broader surface of either palm or sole, has its origin in a raised conical mound, surrounded by an encircling duplicature of skin (Fig. 1). This assemblage of parts becomes covered by series

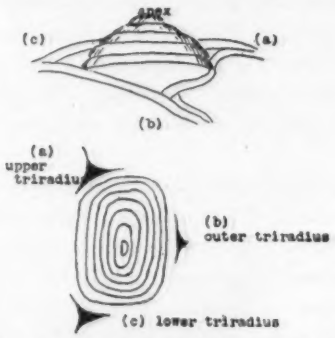


Fig. 1. Diagrams of a pad, and its enclosing folds showing apex, concentric circles and triradii.
Above—Profile view.
Below—Ground plan of same.

of epidermic ridges, themselves composed of rows of single units, probably the morphological equivalents of scales, which in their configuration are influenced by the underlying details. The encircling folds develop as similar ridges which follow the edges, and the two, three, or four points where different folds meet each other form triangular fields, or often triradiate lines diverging from centers, the entire system of which precisely

encloses and defines the pattern areas, upon which the definite patterns are developed. These latter, in typical cases, form in a definite relation to the original conical mound, and develop in concentric circles about the apex of the cone, with the center, or "core," of the pattern exactly coincident with this apex.

When, in the Primates, the height of these structures, that is, folds and mounds, becomes reduced to a flat surface, the relief becomes transformed into a picture, and the mound, with its enwrapping folds, becomes distinctly traceable in the ridges, the pattern area covered with concentric circles, and the outer contour interrupted by triradii. Two radiants of each triradius, extend in nearly opposite directions and embrace, or frame in, a part of the perimeter of the pattern, while a third, the divergent radiant, passes off centrifugally from the pattern.

Those patterns which are located upon the surface of the palms or soles, have each three of these embracing triradii, save the Third of the Interdigital series of the hand, which has four, but the apical patterns, doubtless because of the rounded terminations of the ends of the digits, are furnished with two only, the lateral ones, while the ridges which terminate the digits continue to follow around the contour of the digit, and dispense with a third triradius.

The arrangement of each pattern and its surroundings, including the number of triradii originally embracing each pattern, and their relative position, may be seen by a diagram, published several times, to which the reader is referred.[1] Aside from this the conditions seen in generalized quadrupedal mammals, as shown in the 1904 paper of Miss Whipple,[2] and from which this key diagram was deduced, is of fundamental interest. See especially the paws of *Microtus*, pp. 270 and 272, that of *Crocidura* on p. 280, and the generalized diagram of this author on p. 275, in which each radiant of each triradius is named.

To select a good typical pattern, the transformations of which could be conveniently studied, we may propose the *hypothenar of the hand* (Fig. 2) for the following distinct advantages:

1. It is a pattern with three typical triradii, and consequently three divergents. It is thus better than any finger-print.

[1] "Palm and Sole Studies," BIOL. BULL., Feb., 1916, Figs. 3–5, p. 142–144.

[2] *Zeitschr. f. Morph. u. Anthrop.*, Bd. VII., 1904.

2. It is a large pattern, not placed very near any other, and hence so far beyond the influence of others that we are seldom at a loss to decide whether a certain feature belongs to it or to an adjacent pattern.

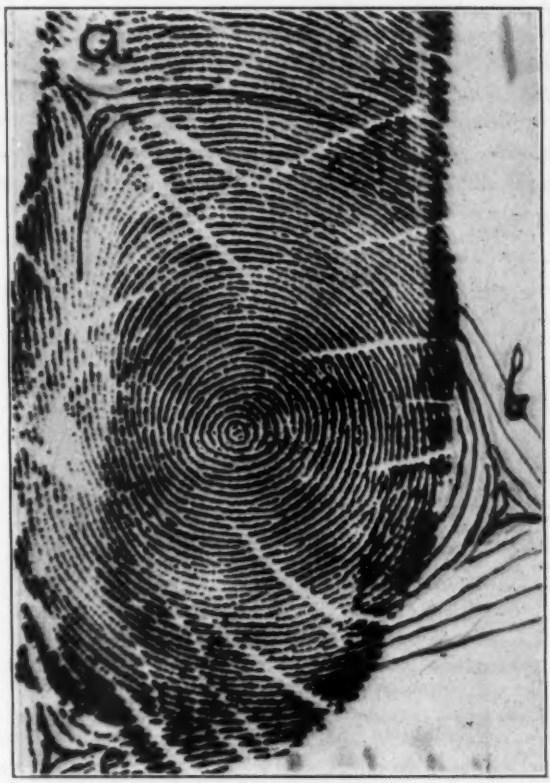


FIG. 2. Actual print of a primitive hypothenar. Triradii *a* and *c* are shown in the print; triradius *b* is indicated by the curve of the friction-ridges and is completed by pencil lines.

3. The region where it occurs is covered by large, heavy ridges, the prints of which are among the most distinct upon the entire palm.

4. This pattern presents an unusual number of variations, and is of fairly common occurrence, rendering it easily possible to collect a large amount of material.

Referring again to any of these diagrams, it will be seen that the pattern in its typical form is centered upon about the middle of the hypothenar region of the hand, and that its three triradii consist typically of (*a*), an ***inner upper***, (*c*), an ***inner lower***, and (*b*), an ***outer***, the first situated near the hollow in the center of the palm, the second down upon the wrist near its middle line, and the third far around upon the outside edge of the hand. This last is, indeed, frequently placed so far around the hand as to be ***extralimital***, that is, placed beyond the limits of the true friction-skin, and indicated only by the convergence of the friction ridges along the transition zone between friction-skin and the normal skin of the back of the hand.

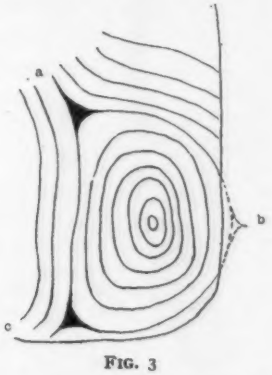


FIG. 3

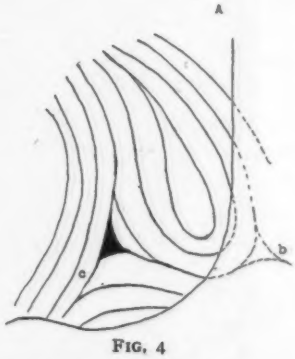


FIG. 4

FIG. 3. A typical hypothenar, based on Fig. 2, an actual print.
FIG. 4. A hypothenar pattern, in which triradius *a* is wanting, and the ridges escape in that direction. This is type *A*.

It is now possible to imagine any one of these three triradii degenerating, and this degeneracy going so far as to allow the escape of the ridges that are normally held in place by its two embracing radiants. Such a loss would convert the concentric circles, a WHORL pattern, into a typical LOOP pattern, open to the point from which the given triradius has disappeared; and as these three triradii are designated as *a*, *b*, and *c*, (Fig. 3) the three resulting loops may receive their names from the triradius that has broken down in each case, and we have types *A*, *B*, and *C*.

In type *A* (Fig. 4) triradius *a* is wanting, while *b* and *c* persist,

thus forming a loop which opens obliquely up across the palm, following the "Line of Life" and pointing up towards the base of the index finger. Type *B* (Fig. 5) opens outwards towards the outer margin, where triradius *b* is wanting, and thus allows the figure to open widely on this side. Triradii *a* and *c* are persistent. In type *C* (Fig. 6) the persistent triradii are *a* and *b*, while the failing one is *c*, the lower medial one. This results in the formation of a loop that runs obliquely downwards and medially, pointing towards the middle of the wrist.

Aside from these three simple types, which result from the loss of a single triradius, there may be found those that result

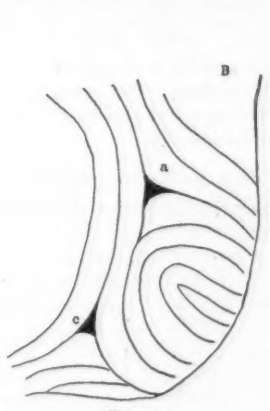


FIG. 5

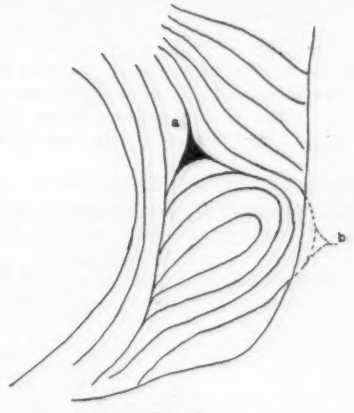


FIG. 6

FIG. 5. A hypothenar pattern, in which triradius *b* is wanting, and the ridges escape in that direction, forming type *B*.

FIG. 6. A hypothenar pattern, in which triradius *c* is wanting, and the ridges escape in that direction, forming type *C*.

from the loss of two out of the three triradii. In these the final result is not a loop, but a lenticular or crescentic figure, tapering at the two ends of a prolonged axis. In type *AC* (Fig. 7) the axis of the figure runs in a curve from above, where it tapers to the hollow of the hand, down to the middle of the wrist, where it tapers again; in type *AB* (Fig. 8) a similar figure is placed obliquely across the hypothenar eminence from upper medial to lower lateral, retaining triradius *c*.

At the present moment I am not quite sure whether type *BC*

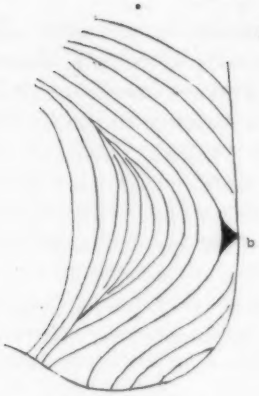


FIG. 7.

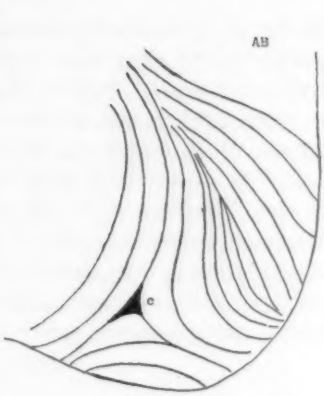


FIG. 8.

FIG. 7. A hypothenar pattern, in which both triradius *a* and triradius *c* are wanting, and the ridges escape in these two directions. This is type *AC*.

FIG. 8. A hypothenar pattern, in which both triradius *a* and triradius *b* are wanting, making type *AB*.

occurs or not, but there seems no reason why it should not. This type (Fig. 9) should retain triradius *a*, the radiants of which

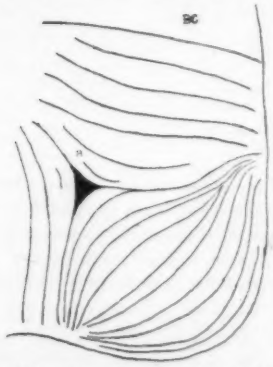


FIG. 9. A hypothenar pattern, in which both triradius *b* and triradius *c* are wanting, forming type *BC*.

should embrace a lenticular figure, shaped like the two others, but with its longitudinal axis running obliquely across the lower outer corner of the palm and tapering down to the points where one normally expects the two triradii, *b* and *c*.

Thus, starting with the primitive whorl, and adding the three

types that arise from the loss of each of the triradii, one at a time, and also the three arising from the simultaneous loss of any two, we have *seven primary types*, in which the variations are due to differences in the originally embracing folds, with their triradii,

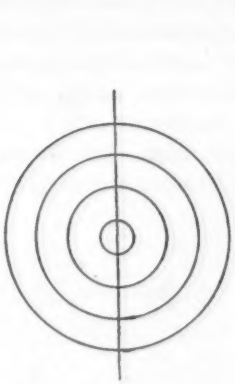


FIG. 10

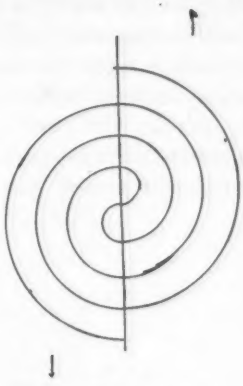


FIG. 11

FIG. 10. A typical whorl pattern, composed of concentric circles. By cutting this through the center, as along the line, and then sliding the two halves as in Fig. 11, an S-shaped spiral is produced.

FIG. 11. An S-shaped spiral the result of sliding the two halves of Fig. 10, as explained in the text. This form is frequent in all kinds of patterns.

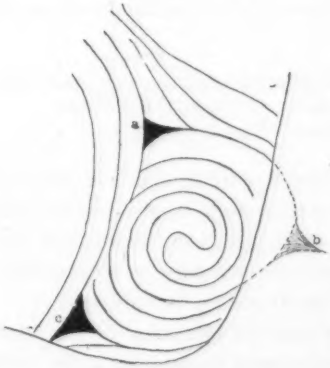


FIG. 12

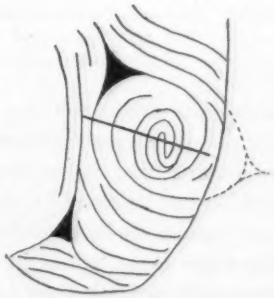


FIG. 13.

FIG. 12. An S-shaped pattern surrounded by its defining triradii.

FIG. 13. An S-shaped pattern, showing the axis along which the slipping may have taken place.

and have nothing to do primarily with the ridges of the pattern itself.

Turning now to the latter there seems to be one distinct line of modification affecting the center of the Whorl; the effect of a shoving of the two halves in opposite directions. This action is most readily seen, and its effect comprehended, by drawing out upon a card a series of concentric circles of the same width, cutting the figure in two through the center, and then slipping the two parts on each other to any distance desired.

Frequently this slipping past of the ridges upon the two sides affects only the central portion of the pattern area, giving a

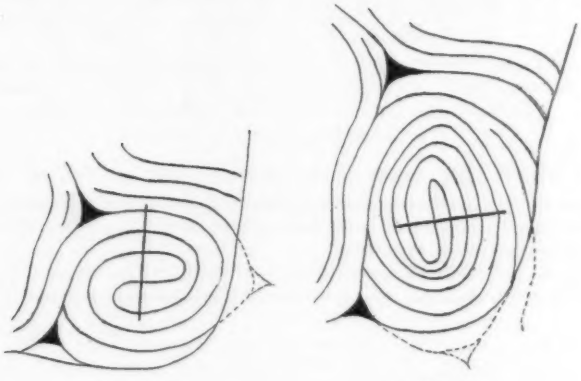


FIG. 14 FIG. 15

FIG. 14. An S-shaped pattern, showing an axis of slipping unlike that of Fig. 13.
FIG. 15. An S-shaped pattern, showing an axis of slipping unlike those shown in Figs. 13 and 14.

result quite like an ordinary Whorl, with an S in the center (Fig. 12). In such cases it remains only to be determined the axis of the slipping, that is, the line along which the diagram of Fig. 10 must be cut in order to slip in such a way as to produce the exact pattern we have before us (Figs. 13, 14, and 15).

In other cases, however, where the extension of the slipping spreads the two half-loops further and further apart, the pattern may become spread out so far that the S-shaped figure covers practically the entire hypothenar area, thus producing an actual double figure, or a figure with two distinct loops, and even a

distinct separation between them. In these cases there may appear a new triradius between the two parts, a "degeneration triradius," resulting from this process, and having no counterpart in any element of the primary pattern or its surroundings (Fig. 16).

The two portions of this outspread pattern seem to develop without any correlation; the upper one may remain as a large loop (Fig. 18) or may disappear (Fig. 17, 19). Similarly the

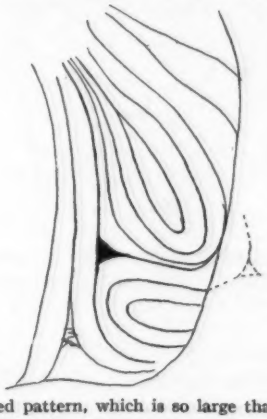
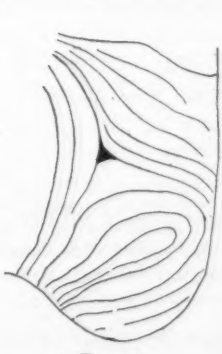


FIG. 16. An S-shaped pattern, which is so large that there has developed a "degeneration-triradius," which cuts the pattern in two and makes two loops of it. Each loop is apparently independent and is directed in different directions.



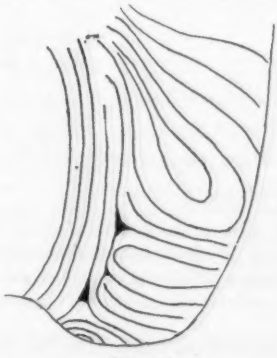


FIG. 17 FIG. 18

FIG. 17. A double S-shaped pattern, in which the loops differ in direction from those shown in Fig. 16.

FIG. 18. A double S-shaped pattern with the two loops again unlike those of Figs. 16 and 17.

lower one may appear as a loop, either facing in (Fig. 17) or out (Figs. 18, and 19). Finally the two parts may both become entirely effaced (Fig. 20) the last sign of the former pattern being the degeneration triradius, with the separation of the area of otherwise parallel lines into the two parts of an original S-shaped pattern.

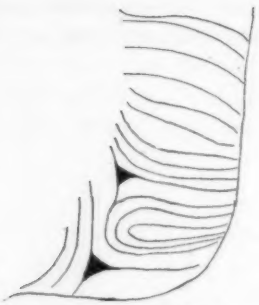


FIG. 19. A double S-shaped pattern, in which the upper loop has disappeared, and is represented by lines that show no trace of a curve.

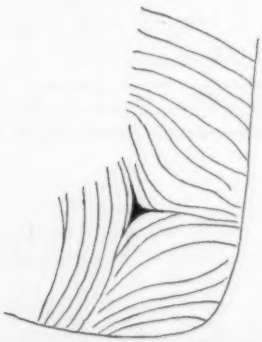


FIG. 20. This shows the ridges covering a hypothenar region, covered by parallel cross-lines, but showing a degeneration triradius, dividing the entire area into halves. It may be supposed that during the evolution of this form it may be evolved from an S-shaped spiral pattern, which has developed a degeneration triradius, and that each pattern of this has degenerated completely.

It is possible to bring into the category of spread-out S-patterns certain cases that consist simply of a rather small and narrow loop, placed very low down on the palm, adjacent to the carpal margin (Fig. 21). This loop suggests the lower half of the double

S pattern, with the loop facing as in Fig. 17, but eventually curving upwards instead of downwards. There may be, in these cases no trace of either a degeneration triradius or of the separation of the whole area into the two halves resulting from an elongated S, and it may be simpler to refer this form to a simple *A* loop of an unusual shape and position.

FIG. 21. In this area there is a degeneration triradius, and the lower pattern has retained its loop. This suggests an explanation for a common type, in which the hypothenar region is made of parallel lines, but has a small loop very low down.

The Migration of Triradii.—Thus far we have contented ourselves with the selection, and somewhat with the classification, of types of patterns, and have separated and grouped the following:

W—The Whorl Type (primitive).

A—The *A*-Type, a loop opening upward.

B—The *B*-Type, a loop opening laterally.

C—The *C*-Type, a loop opening downwards and inwards.

AB—The *AB* Type, a lenticular figure, extending between the positions of triradii *a* and *b*, but with the triradii themselves represented only by converging lines, and with the two embracing radiants wanting.

AC—The *AC* Type, same as before but with the missing triradii *a* and *c*, and represented only by converging lines in the two positions.

BC—The *BC* Type, same as the two foregoing, but with the missing triradii *b* and *c*.

S—The *S*-Type, a whorl with an S in the center. This S figure may be confined to merely a few of the central circles,

or may be more extensive. It may be elongated to cover the entire area, and its axis may be placed at any angle.

D—The Two Loop Type, developed from an elongated S, the two parts of which are separate and distinct from each other, and separated by a "degeneration triradius." In subdivisions of this type the two original loops may develop independently of each other.

It has been suggested by the method of description of these types that triradii do degenerate, commonly forming converging lines without lateral radiants; it is also assumed that they migrate in position. In this latter case the movement may be either *centripetal*, the two lateral radiants approaching the center, or *centrifugal*, the lateral radiants allowing more and more complete rings to intervene between the center or core of the pattern and the center of the triradius. In this latter case the two lateral radiants naturally meet at a sharper and sharper angle until eventually they become lost in the set of converging ridges, and are not to be distinguished from the rest. All stages in these two processes are seen in individual cases, although, as no changes are to be found during the life of an individual the process can only be inferred. Thus to start with a case of centripetal movement we can see this only by finding a series of stages, each permanent for a given individual but representing steps in the process carried on by the race. Whether these steps may be found to be taken phylogenetically along a line of descent is not known as yet.

Thus far, in some 3400 human hands, there has been found an hypothenar pattern that does not belong easily to one of the types described here; I found in a Japanese male a hypothenar that cannot well be thus included, and I wish to present it here without further explanation (Fig. 22). It evidently corresponds closely to the types in which the entire pattern is divided by a "degeneration triradius" into two parts; but where in the most of these, each part may be found in the form of either a loop or a completely degenerated area in which it is covered with a series of parallel ridges without trace of a pattern, the upper half of this one, instead of being a loop as might be expected, is in itself a

perfect Whorl, like the pattern we commenced with while below this there is a complete loop. I have looked at this anomalous pattern again and again, without coming any nearer to any explanation. I have therefore given it as it is, an actual pattern which cannot be explained, and the only one out of several

FIG. 22. A wholly excentric hypothenar region found in the left hand of a Japanese. The pattern is double, but instead of each consisting of a loop, degenerate or otherwise, one of them is a loop while the other is a whorl, the type from which we began in the explanation of all double types. It is possible, on the other hand, that we may have here another explanation in which we have to begin with two whorls, divided by a triradius, like the one given here.

thousands. The explanation may occur to some one who reads this paper, or it may well represent a new type, which will require some new modification of the primary pattern; yet it may hardly lead to the abandonment of the fundamental plan of a pattern, which certainly rests upon too much that is fundamental to suggest so radical a procedure.

THE AXIAL GRADIENTS IN HYDROZOA. VIII. RESPIRATORY DIFFERENCES ALONG THE AXIS IN TUBULARIA WITH SOME REMARKS ON REGENERATION RATE.

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AXIAL DIFFERENCES IN OXYGEN CONSUMPTION.

The experiments recorded in this section were performed at Swan's Island, Maine, in August, 1924. This island is one of a group off the coast of Maine, opposite Rockford, and is directly south of Mt. Desert Island. My visit to Swan's Island was occasioned through information received from the Anglers Company (now the Denoyer-Geppert Company) that *Tubularia* flourishes in that locality during the summer months, at which season, as is well known, it is in poor condition at Woods Hole. The company further kindly invited me to make use of their collecting station and equipment located on Swan's Island. I am greatly indebted to the company in all of these matters and particularly to Mr. Philip Turner, a member of the firm, for assistance in collecting *Tubularia*.

The collecting station at which these experiments were performed is situated in the town of Swan's Island, which town is located at the head of a large harbor and is provided with the usual wharves, pilings, etc. *Tubularia* was found in large quantities on the pilings of the steamer wharf and is apparently restricted to this one habitat. Members of the Anglers Company informed me that they had never seen it in any other situation nor on any other of the wharves or pilings. Large quantities of the hydroid were readily obtained at low tide from the piles supporting the wharf.

The *Tubularia* occurring at Swan's Island does not seem to be identical with the *T. crocea* of Woods Hole. The stems are much more elongated and branching less frequent. These character-

istics may, however, result from the low temperature of the water, which did not, on the warmest summer days, exceed 15° C. The species is very favorable for experimental work, owing to the long unbranched stems and their freedom from other growths.

For the oxygen consumption large colonies were collected and from these a number of long straight clean unbranched stems were selected. They were carefully examined under the microscope to determine that they were free from diatoms or other organisms. In some cases a few such organisms were seen growing on the perisarc but it was found that these could be removed by gently brushing the stem with a camel's hair brush. The hydranth and upper two or three millimeters and the basal part of the stem were then cut off, leaving a clean stem twenty to thirty millimeters long. This was then cut into two pieces, the basal piece generally taken a little longer than the apical piece to compensate for its smaller diameter. Six to eight such pieces were used in each experiment and an attempt was made to select for each experiment stems of similar diameter. All stems used in any one experiment came from the same colony.

The method of determining the oxygen consumption was the same as previously employed in a similar study on *Grantia* (Hyman, '25). For details this paper should be consulted. Briefly small tubes detachable into two sections are employed. At the end of the experiment the pieces of stem are brought by gravity into one section, which is then removed; the other section is analyzed for oxygen content. In each experiment four tubes are employed: one containing the six to eight apical pieces of stem; one containing the basal pieces of the same stems; and two water blanks.

To compare the rate of oxygen consumption of two objects, it is necessary to know the quantity of protoplasm in each. Owing to the lack of a balance at Swan's Island, the weights of the pieces could not be determined. There would be some difficulty in determining the weight, owing to the presence of the lifeless perisarc around the stems; but some method could probably be devised to eliminate this difficulty. Under the circumstances, however, I was compelled to use the volume of the pieces as a standard of comparison. After each experiment

the diameters of the stems were determined under the compound microscope with an ocular micrometer. However uniform a stem may appear to the eye naturally under the microscope considerable variations in diameter are perceived. Some fifteen to thirty measurements of the diameter of the cœnosarc were made at frequent intervals along each stem and these were averaged. The length of the piece was measured on a millimeter rule. From the average diameter and the length the volume of the piece was calculated assuming it to be a cylinder. No correction was made for the central cavity. The volumes of all of the apical pieces in any one experiment were added together and the volumes of the basal pieces similarly; and when the oxygen consumed is divided by these volumes, the quotients can be used to compare the rates of oxygen consumption of apical and basal pieces.

There is no doubt that considerable error is involved in such determinations of the volume of cœnosarc in the pieces. It does not seem to me, however, that the weight of the cœnosarc could be determined any more accurately. All determinations of the rate of respiratory metabolism are necessarily erroneous since there is no known way of discovering the actual quantity of respiring protoplasm in an organism. The consistent results which I have obtained in *Tubularia*, the definite relation noted between level and oxygen consumption, and diameter and oxygen consumption, indicate that the experiments are sufficiently accurate to render the conclusions acceptable.

Nine experiments were performed. The results are presented in Table I. The first column of figures in this table gives the oxygen content in cubic centimeters of the water in the tube at the beginning of each experiment; the second column the oxygen content in the tube at the end; and in the third column is given the difference between the first and second columns, or the oxygen consumed by the pieces. The data are presented in this way for the sake of simplicity; they are not actually obtained in this form as the original oxygen content of the tubes containing the pieces has to be calculated from the blanks. The differences in oxygen content of the two tubes in each experiment at the start are due simply to differences in the volumes of the

tubes; for the same water is used in both tubes in each experiment. The fourth column of figures gives the total volume of the pieces in cubic millimeters, the fifth column the average diameter of the pieces, and the sixth column the oxygen consumed per cubic millimeter in the time occupied by the experiment. Each experiment lasted four hours. In the first experiment six

TABLE I.

SHOWING THE CUBIC CENTIMETERS OF OXYGEN CONSUMED PER CUBIC MILLIMETER OF VOLUME IN FOUR HOURS BY APICAL AND BASAL PIECES OF THE STEM OF *Tubularia*.

Duration of all experiments, four hours.

No. of Exp.	Level of Pieces	O_2 Content at Start, cc.	O_2 Content at End, cc.	O_2 Consumed, cc.	Vol. of Cœnosarc, cu. mm.	Aver. Diam., mm.	O_2 Consumed per cu. mm. in Four Hrs.
4	Apical	.070	.061	.009	32.97	.63	.00027
	Basal	.060	.054	.006	30.84	.59	.00019
5	Apical	.068	.060	.008	24.71	.55	.00032
	Basal	.061	.055	.006	21.90	.53	.00027
3	Apical	.065	.055	.010	20.51	.58	.00048
	Basal	.060	.052	.008	18.68	.46	.00042
6	Apical	.065	.055	.010	20.34	.53	.00049
	Basal	.065	.057	.008	19.61	.51	.00040
7	Apical	.077	.067	.010	18.39	.50	.00054
	Basal	.067	.060	.007	19.25	.48	.00036
9	Apical	.072	.057	.015	20.25	.49	.00074
	Basal	.068	.057	.011	21.76	.51	.00050
8	Apical	.076	.061	.015	17.02	.46	.00088
	Basal	.071	.061	.010	15.08	.44	.00066
1	Apical	.068	.058	.010	11.48	.43	.00087
	Basal	.058	.052	.006	11.10	.41	.00053
2	Apical	.067	.056	.011	13.52	.38	.00081
	Basal	.063	.055	.008	14.59	.35	.00054

pieces of stem were used; in the other experiments eight pieces. The temperature was 20° C. in experiments 1 to 3 and 16° C. in experiments 4 to 9. The volumes of oxygen as presented in the table have been corrected for these temperatures. The nine experiments are arranged in the table in the order of the diameters of the stems used, largest first, smallest last. As already stated,

the six or eight stems in each experiment were selected so as to be of similar diameters.

The following generalizations may be drawn from the data given in Table I.:

1. In all cases the rate of oxygen consumption per unit volume is higher in the apical than in the basal pieces. This result is in harmony with differences in rate of regeneration and in electrical potential which exist along the stem of *Tubularia* (cf. Hyman, '20).

2. There is an inverse relation between the diameter of the stems used and the rate of oxygen consumption, the rate being higher the smaller the diameter of the stems. This agrees with the general law that in animals respiratory rate is inversely proportional to size.

3. In general it appears that the respiratory differences between apical and basal pieces are greater the smaller the diameter of the stem. This indicates that the respiratory gradient is steeper the more slender the stem.

These conclusions are considered further in the discussion.

Experiments on Regeneration.

Data on certain questions regarding regeneration which were obtained on *Tubularia marina* at Pacific Grove, California, in 1922, on *Tubularia crocea* at Woods Hole, Massachusetts, in 1924, and on *Tubularia* sp. at Swan's Island, Maine, in 1924 are herein presented. Owing to the large amount of work that has already been done on the regeneration of *Tubularia*, it does not seem necessary to present these data in detail. For the most part general statements will be made.

The expression "rate of regeneration" is defined and used to mean the time which elapses between the act of cutting and the attainment of a condition of equilibrium. Since in pieces of *Tubularia* of the size employed complete regeneration of a hydranth at the apical end of the piece always occurs, a condition of equilibrium is here synonymous with the completion of a hydranth. Practically, however, it is difficult or impossible to determine the exact time at which the regenerated hydranth is complete. As is well known, the new hydranth forms within the

perisarc and the time of completion of its formation in situ would furnish the most accurate measure of rate of regeneration if such time could be determined. For practical purposes one generally notes the time at which the finished hydranth emerges from the top of the old perisarc. There is no doubt some error in such a procedure for in some cases at least such emergence may be greatly delayed by irregularities in the perisarc through which the hydranth must force its way. However, no better criterion of the time of completion of regeneration has been suggested by anyone and the time occupied by the regeneration process will here be used to signify the time between cutting and the emergence of the completed hydranth.

In all regeneration experiments straight healthy unbranched stems were employed. These were invariably cut as follows. The hydranth and upper millimeter or two and the basal portion of the stem were removed and discarded. The remaining stem was then cut as desired.

The regeneration of only the oral hydranth—*i.e.*, the hydranth which forms at the apical end of each piece—was studied. Unless specifically stated otherwise, all statements refer to this hydranth only. No study was made of the regeneration of aboral hydranths.

1. *Rate of Regeneration of Halves of the Stem.*—In such experiments the stem is divided into halves and the time between cutting and emergence of oral hydranths noted for apical and basal halves. As above stated the observations refer only to regenerated oral hydranths. In a previous paper (Hyman, '20) I presented a considerable mass of data on this matter using *Tubularia crocea*. These experiments showed that the time between cutting and emergence of oral hydranths is markedly shorter in apical than in basal halves. Since writing that paper the same result has been obtained with *T. marina* and with the Maine species. The result on *T. crocea* was also again verified. In Table II, I present a typical experiment on *T. marina*.

2. *Rate of Regeneration of Thirds of the Stem.*—A few experiments on this point were performed on *T. marina* and on the Maine species. The result was found to be different in the two species. In *T. marina*, the apical third regenerates first, the

middle third next, and the basal third last, the differences between the three pieces being well-marked. The basal third in *T. marina* is generally much behind the other pieces. A typical experiment on *T. marina* is given in Table II. On the contrary in the Maine species, the time between cutting and emergence of hydranths is about the same for the middle and basal thirds, the apical third being in advance as usual. I have, however, but five sets of pieces in which the time of emergence of the oral hydranths was exactly determined in all three pieces. These times are as follows, apical third first, middle third next, basal last: 39, 45, 46 hours; 41, 52, 52 hours; 42, 53, 53 hours; 43, 50, 53 hours; and 41, 46, 46 hours. These pieces were 8 to 10 mm. long.

TABLE II.

REGENERATION RATE OF HALVES AND THIRDS OF THE STEM OF *Tubularia marina*.

The first column gives the hours elapsed between cutting and emergence of the oral hydranths; and the other columns the number of hydranths emerged at the hours indicated. Length of halves, 3–7 mm.; length of thirds, 5–8 mm.

Hours Since Cutting.	Halves.		Thirds.		
	Apical.	Basal.	Apical.	Middle.	Basal.
29	1	0	1	0	0
30	3	0	1	0	0
31	6	1	1	0	0
32.5	9	2	3	0	0
33.5	12	4	6	0	0
34.5	14	9	8	0	0
35.5	17	11	9	2	0
37	18	14	10	3	0
39	19	16	10	6	0
41.5		18	10	6	0
43		19	10	9	1
44.5			10	9	3
45.5			10	10	4
46.5			11	10	4
48.5				10	6
50.5				11	6
52.5					8
54.5					9
56.5					11

It thus appears that the physiological differences along the stem which are responsible for the differences in regeneration rate at different levels extend further down the stem in *T. marina* than in either of the Atlantic coast species. Banus ('18)

had previously shown a lack of difference in rate of regeneration of middle and basal thirds in *T. crocea*. Differences in the growth habits of the species are in harmony with the experimental findings. In *T. marina* (see illustration in Child, '15, p. 90), there are no branches but the base of each stem runs along the substratum as a stolon for some distance, then turns vertically, and gives rise to a new hydranth at its tip. This method of growth indicates that the hydranth in *T. marina* dominates a considerable length of stem. The Atlantic coast species, on the other hand, branch freely, frequently at a relatively short distance from the terminal hydranth. This fact suggests that the dominance of the terminal hydranth does not extend very far proximally. Beyond this limit the axial differences along the stem would be slight or absent. It it probable that if shorter distal pieces of the stem of *T. crocea* were cut into thirds, a difference would be found in the regeneration rate of the middle and basal thirds. In both my and Banus' experiments, however, rather long stems were used.

3. *Relation of Rate of Regeneration to the Length of the Piece.*— In a previous paper ('20) I reported that the length of the piece has little effect on the rate of regeneration when diameter and level of the distal cut are constant, except when the pieces are very short. This result was again verified on *T. crocea* and on the Maine species. Thus in 16 pairs of pieces of *T. crocea*, the two of each pair being 10 and 5 mm. long, respectively, with the distal cut taken at the same level in both, the 5 mm. pieces regenerated oral hydranths first in eight cases, the 10 mm. first in eight cases. It is understood that the diameter of the apical end was the same in the two members of each pair. At Swan's Island an experiment was performed comparing the rates of regeneration of pieces 15, 10 and 5 mm. long, the distal diameter and level of the distal cut being the same in the three pieces of each set. The rate of regeneration of the 5 and 10 mm. pieces was equal throughout this experiment; and this was also the case with most of the 15 mm. pieces, but a few of them preceded in production of hydranths the shorter pieces by a short time interval.

When the pieces are shorter than 5 mm. in length the production of hydranths is greatly slowed down, so that the regeneration

of pieces 2 mm. in length, for instance, runs far behind that of longer pieces.

4. *Relation of Rate of Regeneration to the Diameter of the Stem.*—It was previously reported that the time between cutting and emergence of oral hydranths is noticeably shorter the more slender the stem. This was again verified on the Maine species. It is reasonable to believe that this result is correlated with the fact, given in the first part of this paper, that the rate of oxygen consumption is higher, the less the diameter of the stem.[1]

5. *Relation of Size of the Regenerated Oral Hydranth to the Length of the Stem.*—A number of measurements were made both at Woods Hole and Swan's Island of the size of the regenerated hydranths on stems of the same distal diameter and cut at the same distal level but of different lengths. Measurements were made with an ocular micrometer, and on the fully expanded hydranth some hours after emergence. The length of the tentacles, the length of the body (distance from tip of manubrium to constriction just below base of hydranth), and width of the widest part of the hydranth (part bearing the proximal tentacles) were measured. The most extensive experiment of this kind was done at Swan's Island. A number of pieces having the same apical level but of different lengths and diameters were cut and placed in one bowl. After regeneration the dimensions of the regenerated oral hydranths and of the original piece were taken. These data are given in Table III. and are arranged with reference to the diameter of the apical end of the piece.

These data show that the dimensions of regenerated oral hydranths are about the same on pieces of quite different lengths but of the same diameter and with their apical ends at the same level. This statement would probably not apply to very short pieces (under 2 mm.) but as shown in the table pieces as short as 2 mm. may regenerate hydranths as large as those from pieces several times as long. Some of the longest pieces may produce

[1] Stems whose diameter is smaller are of course also smaller in other particulars—total length and size of hydranths. As pointed out later they are simply younger as a rule than larger stems. It seems convenient to take the diameter of the stem as a measure of age. Wherever it is stated throughout this paper that the diameter of the piece is smaller it is to be understood that such pieces were taken from stems of general small proportions.

hydranths slightly larger than much shorter pieces but this difference is slight at best and in no wise proportional to the great differences in length of such pieces.

TABLE III.

DIMENSIONS OF REGENERATED ORAL HYDRANTHS WITH REFERENCE TO LENGTH OF PIECE.

Length in millimeters; other figures units of ocular micrometer.

Length of Piece, mm.	Diameter at Apical End of Piece.	Length of Body of Hydranth.	Width of Body of Hydranth.	Length of Tentacles.
2	6.0	15	6	12
5	6.0	18	8	11
9	6.0	21	10	15
2	6.5	14	8	10
5.5	6.5	20	9	13
5	6.5	15	7	12
15	6.5	24	11	17
15	6.5	20	8	14
2.5	6.8	19	8	14
5.5	6.8	22	9	14
5.5	6.8	20	7	10
10.5	6.8	23	12	18
17	6.8	23	12	15
2	7.0	20	7	16
5.5	7.0	18	8	12
5.5	7.0	19	8	13
6	7.0	19	9	14
10	7.0	22	9	14
10	7.0	23	10	15
10	7.0	25	11	15
10.5	7.0	19	9	14
11	7.0	18	8	12
5	7.2	19	8	11
15	7.2	24	10	13
5	7.5	19	10	15
5	7.5	20	9	12
5.5	7.5	20	9	12
11	7.5	20	10	15
11.5	7.5	24	12	22
16	7.5	27	12	19
2.5	8.0	21	8	15
10	8.0	25	12	17
2.5	8.5	22	9	11
5.5	8.5	24	12	12
5.5	8.5	22	9	12
10	8.5	22	11	16

6. *Relation of Size of the Regenerated Oral Hydranth to the Diameter of the Stem.*—Some experiments were performed on this matter. It was found that the size of the hydranth is slightly larger in stout than in slender stems when other factors are eliminated. This difference is detectable only when the difference in diameter of the stems is considerable. In Table III., where there are but small differences in diameter, no definite relation between dimensions of regenerated oral hydranths and diameter of the stems appears. But when stems differing markedly in diameter are compared the dimensions of the regenerated oral hydranths are seen to bear some slight relation to the diameter of the oral end of the pieces. One experiment of this kind is given in Table IV. Stout and slender stems from the same colony, of the same length, and cut at the same apical levels were allowed to regenerate and the resulting oral hydranths measured as in the preceding section. There were ten stems of each lot; these have been averaged in the table for brevity.

TABLE IV.

DIMENSIONS OF REGENERATED ORAL HYDRANTHS WITH REFERENCE TO THE DIAMETER OF THE APICAL END OF THE ORIGINAL STEMS.

Ten pieces in each lot, pieces 20 mm. long. Figures, units of the micrometer scale.

	Diameter Apical End.	Length Body of Hydranth.	Width Body of Hydranth.	Length Tentacles.
	Lot of Slender Stems.			
Min.	5.5	19	8	15
Max.	7.0	26	11	21
Aver.	6.2	23	10	17
	Lot of Stout Stems.			
Min.	7.5	22	11	18
Max.	11.0	30	15	22
Aver.	8.7	25	12	19

Table IV. shows that in the stouter stems the dimensions of the hydranth are slightly larger on the average than in the slender stems. The chief difference is in the width of the hydranth.

The result is readily understandable when it is recalled that in the regeneration of *Tubularia*, the new hydranth is laid down in the old cœnosarc. The new hydranth will then be necessarily broader the stouter the stem.

It will be perceived that the differences in dimensions of regenerated hydranths on stems of different diameter are in no wise proportional to the diameters. In Table IV., the diameters differ by 40 per cent. on the average, while the differences in dimensions of the regenerated hydranths average 10 to 20 per cent. Further, the more slender stems regenerate in a shorter length of time.

7. *Relation of the Size of the Regenerated Oral Hydranth to the Level of the Stem.*—In determining this matter it is necessary that the diameter of the apical ends of the pieces to be compared be the same, for, as shown above, diameter affects the dimensions of the regenerated hydranth. It is a little difficult to obtain pieces from different levels of the same apical diameter since in *Tubularia* the stem generally tapers towards the base. However, it is occasionally possible to find stretches of stem of approximately the same diameter throughout or even some which increase in diameter proximally. Only such have been used in making the comparison. From such stems apical and basal pieces of equal length were cut and after regeneration the dimensions of the regenerated oral hydranths determined. Some data of this kind, obtained at both Woods Hole and Swan's Island are given in Table V.

It is obvious to the eye and measurements also demonstrate that in pieces of the same diameter and length but taken from different levels, the dimensions of the regenerated oral hydranth are nearly always greater on the apical than on the basal piece. Level is thus the most important factor in determining the dimensions of regenerated oral hydranths. These size differences of oral hydranths also of course appear in pieces cut from the usual type of stem, where the diameter of the basal piece is smaller than that of the apical piece; and are too great to be accounted for merely on the differences in diameter.

TABLE V.

DIMENSIONS OF REGENERATED ORAL HYDRANTHS ON PIECES OF THE SAME LENGTH AND DISTAL DIAMETERS BUT FROM DIFFERENT LEVELS.

Figures, units of the micrometer scale.

Level of Pieces.	Diameter Distal End.	Length Body of Hydranth.	Width Body of Hydranth.	Length Tentacles.
Apical.....	10.0	15	7	20
Basal.....	10.0	12	7	16
Apical.....	5.0	14	5	15
Basal.....	5.0	10	2	7
Apical.....	6.5	20	10	23
Basal.....	6.5	16	7	18
Apical.....	5.5	30	9	23
Basal.....	5.5	17	8	27
Apical.....	7.1	20	10	25
Basal.....	7.1	17	9	18
Apical.....	6.6	27	11	45
Basal.....	6.8	24	11	45
Apical.....	6.4	28	10	40
Basal.....	6.4	23	10	35
Apical.....	6.3	23	7	27
Basal.....	6.3	21	7	12

DISCUSSION.

The foregoing facts together with others available from the literature support the conception of the existence of a metabolic gradient along the main axis in *Tubularia* and other lower forms; and of a relation between this gradient and the rate of regeneration.

It is shown in this paper that the rate of oxygen consumption in the stem of *Tubularia* is higher in apical than in basal levels. It thus appears that there exists a gradation in respiratory rate along the stem of *Tubularia*. Similar respiratory gradients along the main axis were previously reported for other lower invertebrates: *Corymorpha*, *Grantia*, *Planaria*, several annelids (Hyman, '22, '23, '25, Hyman and Galigher, '21). It is reasonable to believe that they are of universal occurrence among at least the lower Metazoa. We believe that such differences in

rate of chemical activity (with which are doubtless associated other graded differences) constitute the basis of the phenomenon of polarity.

It appears further that there exist in these organisms permanent electric currents whose direction of flow bears a definite relation to the respiratory gradient (for data on *Tubularia*, see Hyman, '20, for other hydroids, Hyman and Bellamy, '22, Lund, '22).[2] This relation is the following: any part of the organism is electronegative (in the external circuit) to any part having a lower respiratory rate than itself. It is probable that the gradation in rate of chemical activity is the chief cause of the electrical gradient. Some biologists are of the opinion that these electric currents constitute a tool, so to speak, which enables one part of an organism to affect another part.

There is some indication that in the hydroids the gradient is steeper in the more apical levels and gradually flattens out basally. This inference is drawn chiefly from the electrical data, the potential difference being greatest in apical levels (Hyman and Bellamy, '22, pp. 332–33, Lund, '22, p. 490); but in *Corymorpha* the respiratory evidence is to the same effect (Hyman, '22). Regardless of the slope of the gradient in distal levels, it appears certain from electrical and other data that the gradient is slight or absent or even reversed in the proximal levels of hydroid stems (see references just given). It follows that at a certain distance from the apical end, the primary gradation practically disappears and new gradations running in the same or the reverse direction may be initiated. The distance to which the primary gradation extends coincides with the limits of the individual and beyond this point buds, zooids, etc., may arise, if the constitution of the protoplasm permits asexual reproduction; or if asexual reproduction is impossible, the basal or caudal parts of the organism may be more or less independent physiologically or nervously of

[2] In a later paper ('25) Lund reversed his statement in the 1922 paper as to the direction of the current in *Obelia*, without offering any explanation of the contradiction. It may be pointed out that Hyman and Bellamy ('22) tested the P. D. along the main axis of colonies of a species of *Obelia* common at Friday Harbor and identified by Professor Nutting as *Obelia borealis* and of *Obelia geniculata* at Woods Hole; and in both species found distal levels electronegative to proximal in agreement with the statement in Lund's 1922 paper but contrary to his statement in 1925.

anterior levels. The existence of an oral or apical end inhibits the formation of any other oral or apical end within the distance limit over which the control of the former extends (see Child, '15, Chapter IV.).

Differences in rate of regeneration (time between cutting and completion of oral or apical structures) with respect to level constitute further evidence of the existence of a metabolic gradient in hydroids. The more apical the level in the whole within the limits of the primary gradient from which the piece is taken the more rapidly does it produce a new apical end. This generalization has been shown to hold in a large number of lower invertebrates, mostly cœlenterates, *e.g.*,—*Eudendrium* (Goldfarb, '07), *Tubularia* (Driesch, Morgan, Child, etc., for references see Hyman, '20), *Corymorpha* (Torrey, '10), *Pennaria* (Gast and Godlewski, '03), *Obelia* (Billard, '04, Lund, '23), *Cerianthus* (Child, '03), *Planaria* (Child, '11), annelids (Hyman, '16). Further proof of the correctness of this generalization with regard to *Tubularia* is presented in this paper. It is also shown that such axial differences in rate of regeneration are independent of size or mass differences at different levels. It can scarcely be doubted that the metabolic gradient is the direct or indirect cause of the apico-basal gradation in rate of regeneration of oral or apical structures.

Another instance of the dependence of regeneration rate on metabolic rate is the difference in these regards between organisms of different ages (sizes). It is shown in this paper and previously (Hyman, '20) that in *Tubularia* the rate of regeneration is more rapid the smaller the diameter of the piece; and further that the rate of oxygen consumption is higher the smaller the diameter. There is thus a correlation between rate of production of oral hydranths and rate of respiratory metabolism; and it is scarcely to be doubted that the latter is the direct cause of the former. It is probable that in *Tubularia* the dimensions of the stem (diameter, total length, size of hydranth) vary inversely with age and that we are really dealing here with age and not size differences. An inverse relation between respiratory rate and age (size) appears to be universal throughout the animal kingdom. In a previous paper (Hyman, '19) I reviewed this matter and

quoted a considerable body of evidence in support of this generalization. Since then I have obtained additional evidence (unpublished) of the inverse relation between respiratory rate and size in *Corymorpha*, starfish, nudibranchs, and tadpoles; and other data have appeared in the literature (*e.g.*, Smith, '25). The relation here found in *Tubularia* between rate of regeneration and age probably also is of general application. Przibram ('07) gives a discussion of this matter and reaches the generalization that the rate of regeneration is more rapid the younger the animal and declines with age.

Not only is there a relation between rate of regeneration (time between cutting and completion of oral or apical structures) and respiratory rate but the amount of tissue regenerated in a given time appears also to be dependent upon metabolic rate. This applies both to axial differences and age differences. In general the more apical the level from which the piece is taken the larger is the size of the oral or apical end regenerated, and the greater the total mass of regenerated tissue. This result cannot be ascribed to differences in mass of pieces from different levels for it also holds when the pieces are of equal mass. It is shown in this paper that in *Tubularia*, the regenerated oral hydranth is larger on apical than basal pieces, when the length and apical diameters of such pieces are the same. Driesch ('99) and Child ('07) had previously noted that the length of the primordium of the oral hydranth is greater the more apical the piece. A similar relation between level and the size of regenerated apical structures or total amount of tissue regenerated was observed by Billard ('04) in *Obelia*, Child ('03) in *Cerianthus* and *Planaria* ('11) and Morgulis ('07) in annelids.

The amount of tissue regenerated in a given time is also greater the young (smaller) the organism, relative to its size. This is shown to be the case in *Tubularia*, pieces from smaller stems producing relatively larger hydranths in a shorter time than pieces of equal length from large stems. A similar relation between age and rate of formation of new tissue was found by Zeleny ('07), Ellis ('08), and Scott ('09).

A third factor appears to be involved as regards the amount of tissue regenerated in a given time. This factor is the degree

of injury relative to the mass of the regenerating piece or organism. Some years ago under the leadership of Zeleny there was considerable interest in this matter. A number of papers dealing with this subject were published (Zeleny, '03, '05*a*, '05*b*, '07, Ellis, '07). A general agreement was reached by the workers in this field that any one part is replaced at a more rapid rate, the greater the amount of tissue removed at the original operation. It is possible that this result also depends on metabolic rate; for every wound is the locus of an increase in metabolic rate and the greater the number of wounds and the smaller the mass of tissue remaining the greater is the stimulation of respiratory metabolism not only at the wounds but also in the adjacent uninjured parts.

In general, then, it appears that the size of regenerated oral or apical structures and the amount of tissue produced in a given time are causally related to the metabolic rate of the regenerating mass.

When metabolic factors such as level, age, or wound stimulation are not involved, the mass of the piece appears to have little or no effect upon the amount of tissue regenerated in a given time. Thus it is shown in this paper for *Tubularia* that pieces differing considerably in length and hence total mass regenerate oral hydranths of equal size in equal lengths of time. Because of this lack of relation between mass and regeneration it can be stated as a generalization that the smaller the original mass (within certain limits of course) the greater relative to its mass is the amount of tissue produced in a given time. Other data in support of this statement will be found in many of the papers already cited.

It remains to consider a paper by Lund ('23) on regeneration in *Obelia*, in which paper certain conclusions are stated which seem to be at variance with those presented here. In the regeneration of *Obelia* as previously noted by Billard ('04) a process grows out from the cut surface and the hydranth differentiates at the end of this outgrowth. Lund has studied the time occupied by this growth process and the rate of elongation of the outgrowth in a series of pieces cut in apico-basal sequence from the main stem of colonies of *Obelia*. Lund finds as did Billard

('04) that the time between cutting and completion of the polyp is shorter the more apical the piece. But according to Lund the time between the beginning and end of the outgrowth is the same at all levels. By defining the regeneration period as the time period during which the outgrowth is elongating, Lund is able to reach the conclusion that the "rate of regeneration" is the same at all levels. It may merely be pointed out that adopting some particular definition in no wise alters the facts of the matter, which are the same for *Obelia* as for other cœlenterates. Lund is able to state that the rate of regeneration does not differ at different levels only because his definition of the expression "rate of regeneration" differs from that used by other workers. The point raised by Lund that the time interval from the beginning to the end of the elongation process is the same at all levels may be correct but it does not seem to me to be proved by his tables and graphs. However, it is difficult to come to any decision on the matter, as neither the time of beginning of growth of the apical pieces nor of completion of growth of the basal pieces is given. It is very probable, nevertheless, that the chief differences in regeneration rate at different levels lie in the early part of the regeneration period. It is admitted by Lund and is shown by his tables and graphs that the rate of elongation of the outgrowth is faster the more apical the level. Billard ('04) had previously made a similar observation; he noted that the sum of the lengths of the outgrowth at both ends of each piece is greater the more apical the piece. In the face of his own data, Lund still attempts to maintain the conclusion that the rate of elongation is the same at all levels on the assumption that the rate of elongation decreases apico-basally because the mass of the pieces decreases in the same direction. He assumes without any proof whatever that the amount of tissue regenerated is proportional to the mass of the piece. Such an assumption is incorrect. As already pointed out considerable differences in length of pieces have no effect on either the time required for regeneration or the amount of tissue produced in that time; and such slight differences in amount regenerated as are correlated with differences in diameter are counterbalanced by the more rapid rate of regeneration of pieces of smaller diameter. All of the available

evidence indicates that the mass of regenerated tissue is not proportional to the original mass of the piece but to the contrary is relatively greater the smaller the latter. In view of all of the facts it is practically certain that the apico-basal sequence in rate of elongation observed in *Obelia* pieces is the result of differences in level. Even though the time occupied by the elongation process may be the same at different levels, as insisted by Lund, still the length of outgrowth produced in that time is greater the the more apical the piece; and hence the "rate of regeneration," even using this expression as defined by Lund, decreases apico-basally in *Obelia* as in other lower forms.

Summary.

1. The rate of oxygen consumption per unit volume of cœnosarc is greater in apical than in basal halves of distal regions of the stem of *Tubularia*.

2. The rate of oxygen consumption per unit volume of cœnosarc is greater the younger the stem (smaller its diameter).

3. The time between cutting and completion of oral hydranths is shorter the more apical the piece in pieces of equal length from distal levels of the stem of *Tubularia*.

4. The statement in 3 may or may not hold for proximal regions of the stem, depending on the species.

5. The time between cutting and completion of oral hydranths is independent of the length of the piece when the apical end of the pieces is taken at the same level, except in very short pieces.

6. The time between cutting and completion of oral hydranths is shorter the younger the stem (smaller the diameter).

7. The size of the regenerated oral hydranths is almost entirely independent of the length of the piece, when the apical end of the pieces is taken at the same level, except in very short pieces.

8. The size of the regenerated oral hydranth is slightly smaller especially as to width the smaller the diameter of the stem but not proportionally smaller.

9. The size of the regenerated oral hydranth is absolutely larger on apical than on basal pieces of equal mass taken from distal regions of the stem.

10. In general there is a relation between respiratory rate and

regeneration. The higher the respiratory rate the shorter is the time interval between cutting and completion of oral hydranths and the larger is the size relatively or absolutely of the regenerated oral hydranth.

BIBLIOGRAPHY.

Banus, M. Garcia.

'18 Is the Theory of Axial Gradient in the Regeneration of *Tubularia* Supported by Facts? Jour. Exp. Zoöl., Vol. 26, pp. 265–75.

Billard, A.

'04 Contribution a l'étude des Hydroides. Annal. d. Sci. Natur., ser. VIII, Vol. 20, pp. 1–250.

Child, C. M.

'03 Form Regulation in *Cerianthus*. II. The Effect of Position, Size, and other Factors on Regeneration. BIOL. BULL., Vol. 5, pp. 304–19; Vol. 6, pp. 1–12.

'05 Form Regulation in *Cerianthus*. IX. Regulation and Proportions. Same, Vol. 8, pp. 271–89.

'07 An Analysis of Form Regulation in *Tubularia*. II. Differences in Proportion in the Primordia. Arch. f. Entw'mech., Vol. 23, pp. 415–43.

'11 Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. I. The Axial Gradient in *Planaria dorotocephala* as a Limiting Factor in Regulation. Jour. Exp. Zoöl., Vol. 10, pp. 265–320.

'15 Individuality in Organisms. University of Chicago Press.

Driesch, H.

'99 Studien über das Regulationsvermögen der Organismen. II. Quantitative Regulationen bei der Reparation der Tubularia. Arch. f. Entw'mech., Vol. 9, pp. 103–36.

Ellis, Max M.

'07 The Influence of the Amount of Injury upon the Rate and Amount of Regeneration in *Mancasellus macrourus* (Garman). BIOL. BULL., Vol. 13, pp. 107–13.

'08 Some Notes on the Factors Controlling the Rate of Regeneration in Tadpoles of *Rana clamata* Daudin. BIOL. BULL., Vol. 14, pp. 281–83.

Gast, R., and Godlweski, E.

'03 Die Regulationserscheinungen bei *Pennaria cavolinii*. Arch. f. Entw'mech., Vol. 16, pp. 76–116.

Goldfarb, A. J.

'07 Factors in the Regeneration of a Compound Hydroid *Eudendrium ramosum*. Jour. Exp. Zoöl., Vol. 4, pp. 317–57.

Hyman, L. H.

'19 Physiological Studies on *Planaria*. III. Oxygen Consumption in Relation to Age (Size) Differences. BIOL. BULL., Vol. 37, pp. 388–403.

'20 The Axial Gradients in Hydrozoa. III. Experiments on the Gradient of *Tubularia*. BIOL. BULL., Vol. 38, pp. 353–403.

'22 Oxygen Consumption with Respect to Level, Size, and Regeneration, and Electrical Polarity in *Corymorpha palma*. Proc. Amer. Soc. Zoöl., Anat. Rec., Vol. 24, p. 392.

'23 Physiological Studies on *Planaria*. V. Oxygen Consumption of Pieces with Respect to Length, Level, and Time after Section. Jour. Exp. Zoöl., Vol. 37, pp. 47–68.

'25 Respiratory Differences along the Axis of the Sponge *Grantia*. BIOL. BULL., Vol. 48, pp. 379–88.

Hyman, L. H., and Bellamy, A. W.

'22 Studies on the Correlation between Metabolic Gradients, Electrical Gradients, and Galvanotaxis. I. BIOL. BULL., Vol. 43, pp. 313–47.

Hyman, L. H., and Galigher, A. E.

'21 Direct Demonstration of the Existence of a Metabolic Gradient in Annelids. Jour. Exp. Zoöl., Vol. 34, pp. 1–16.

Lund, E. J.

'22 Experimental Control of Organic Polarity by the Electric Current. II. The Normal Electrical Polarity of *Obelia*. A Proof of its Existence. Jour. Exp. Zoöl., Vol. 36, pp. 477–94.

'23 Same, III. Normal and Experimental Delay in the Initiation of Polyp Formation in *Obelia* internodes. Jour. Exp. Zoöl., Vol. 37, pp. 69–87.

'25 Same, V. The Nature of the Control of Organic Polarity by the Electric Current. Jour. Exp. Zoöl., Vol. 41, pp. 155–90.

Morgulis, S.

'07 Observations and experiments on regeneration in *Lumbriculus*. Jour. Exp. Zoöl., Vol. 4, pp. 549–74.

Przibram, H.

'07 Experimental-Zoologie. II. Regeneration.

Scott, G. G.

'09 Regeneration in *Fundulus* in Relation to the Size of the Fish. BIOL. BULL., Vol. 17, pp. 343–53.

Smith, H. M.

'25 Cell Size and Metabolic Activity in Amphibia. BIOL. BULL., Vol. 48, pp. 347–78.

Torrey, H. B.

'10 Biological Studies on *Corymorpha*. IV. Budding and Fission in Heteromorphic Pieces and the Control of Polarity. BIOL. BULL., Vol. 19, pp. 280–301.

Zeleny, C.

'03 A study of the rate of regeneration of the arms of the brittle star, *Ophioglypha lacertosa*. BIOL. BULL., Vol. 6, pp. 12–17.

'05*a* Compensatory Regulation. Jour. Exp. Zoöl., Vol. 2, pp. 1–102.

'05*b* The Relation of the Degree of Injury to the Rate of Regeneration. Jour. Exp. Zoöl., Vol. 2, pp. 347–69.

'07 The Effect of Degree of Injury, Successive Injury, and Functional Activity upon Regeneration in the scyphomedusan, *Cassiopeia xamachama*. Jour. Exp. Zoöl., Vol. 5, pp. 265–74.

THE VALUE OF INSTINCT AS A TAXONOMIC CHARACTER IN SPIDERS.

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(Contribution from the University of Porto Rico.)

The study of animal behavior under experimental conditions has brought into sharp opposition the supporters of the idea of the rigidity of instinct with the defenders of its plasticity. The inability of the animal, especially of invertebrates to meet new and unaccustomed conditions in human fashion has furnished the chief argument in favor of the attitude of the modern school of behaviorists. The interpretation of an animal as a machine activated by stimuli to reflexes with an outward appearance of intelligence, yet without intelligent control of its actions has been and still is sufficiently often discussed by both parties not to need further discussion here. Some day I hope to put together for print some observations on the plasticity of instinct in spiders, which I have made gradually during the past years. For the present I want to content myself with another aspect of the problem.

The discussion referred to above naturally centers round the phenomena of *individual* behavior. Ascending from individual to species and widening our observations to different geographical regions and environmental conditions, influenced by our preference for the one or the other school of behaviorism we lay stress either on the individual modifications or the specific similitudes of instinct. The same applies of course to generic and family characteristics. The instincts of spiders are apparently particularly rigid in this respect and easily traceable to family relationship in burrowing habits, snare construction, courtship, mating and care of cocoon. Thus all representatives of the family Argiopidæ build orbwebs, all Lycosidæ carry the cocoon attached to their spinnerets, etc. In fact in the earlier days of arachnology the habits were used for family or larger group dis-

tinction and spiders were divided into Terricolæ, Tubicolæ, Citigradæ, Saltigradæ, Laterigradæ, Retithelæ, and Orbithelæ. It was soon recognized, however, that orbwebs are constructed by some spiders anatomically very different from Argiopidæ and since separated from the latter into a family Uloboridæ among the supposedly more primitive division of Cribellatæ. The same was done for the Dictynidæ among the Cribellatæ, which were originally placed with the Retithelæ. The similarity in web construction was explained by the assumption of parallelism in both groups. Similar though not such pronounced parallelism was found in the case of various Tarantulæ or Theraphosid spiders, the habits of which are comparable with those in true spiders. Parallelism of such kind may be easily explained as adaptive to similar conditions of life. But it would be difficult to conceive the construction of such a complicated and perfect snare as an orbweb as a manifestation of habits acquired independently by every genus of Argiopid spiders. The general features of this habit must have been acquired before the splitting up of the original stock into new species and persist notwithstanding the fact that the family spread all over the world from the tropical rain forests to the cold zones of the North and South, and comprises an enormous number of species as unlike each other in size and appearance as *Nephila* with its inch and a half long body and four-inch spread of legs and *Theridiosoma* scarcely a tenth of an inch in length, *Gasteracantha* with her abdomen much wider than long and adorned in some species with long, curved spines exceeding many times the length of the body and *Tetragnatha* with her soft abdomen often many times longer than wide and legs so drawn out and kept in a peculiar fashion that the spider in its web has the appearance of a little twig. All these modifications of structure must have therefore appeared later and influenced only the subordinate features of the habit, the details of the general plan of structure of the geometric web. It was this among other considerations that inclined me to the view which I still hold that the Argiopidæ are descendants of Uloboridæ.

The manner in which the female takes care of the cocoon with eggs is another habit characteristic of whole families. In fact so

distinctive is this habit in some families that on the strength of it the great French arachnologist Simon placed the genus *Rhoicinus* among the Lycosidæ, although neither the disposition of the eyes, nor other important morphological structures are of the type to warrant the inclusion of the former in the very characteristic family of ground spiders. Says Simon in his "Histoire Naturelle des Araignées": "Les quelques espèces, pour lesquelles j'ai proposé le genre *Rhoicinus*, sont très anormales pour la famille des *Lycosides*, dont elles n'ont pas la disposition oculaire; si l'on ne tenait compte que de ce caractère, on les rapprocherait des *Cybaeinæ*, particulièrement des *Campostichomma*, mais leurs trochanters sont entaillés d'une profonde échancrure apicale, leur griffe impaire ne porte q'une seule petite dent basale *et, de plus, j'ai surpris l'une des espèces portant aux filierès son cocon globuleux, comme l'aurait fait un Lycosa.*" (Italics are mine, A. P.) Without discussing the case in question since our knowledge of *Rhoicinus* is as yet very imperfect and I have no first-hand acquaintance with the genus, I merely wish to point out that of the characters mentioned by Simon the notch in the trochanters is a character occurring in other families besides the Lycosidæ, such as Pisauridæ, Clubionidæ, Argiopidæ, etc., the number of teeth in claws is very variable, changing in other families from genus to genus, while the disposition of the eyes is undoubtedly a very old character and remarkably persistent within families, being at the same time very little affected by age. Thus the deciding character in this case remains the cocoon-carrying habit and the question arises, which character is more subject to modification, a fundamental, old morphological character or a certain habit?

On one of my excursions in Porto Rico I was fortunate enough to make an observation which proves definitely that at least the cocoon-carrying habit may be acquired by spiders the morphological characters of which prevent their inclusion in the family Lycosidæ beyond any possibility of dispute. While turning over a rock in a field near Aguas Buenas I noticed two little spiders on the underside of the rock, carrying their cocoons Lycosa-fashion, attached to the spinnerets. Both proved to be new species to which I have given respectively the names of *Bathy-*

phantes ovigerus and *Lithyphantes oöphorus*. The detailed descriptions of the species will be given later in another place, in my study of Porto Rican spiders. *Bathyphanthes* is a well-known genus of the family Linyphiidæ and has numerous representatives in the United States, Europe and other countries. *Lithyphantes* belongs to the family Theridiidæ and has also a wide distribution. Both are small spiders, *B. ovigerus* measuring 2.87 mm. in length, *L. oöphorus* 1.628 mm. Of the former species I have collected later several females and a male under similar conditions not far from Rio Piedras, of the *Lithyphantes* a second female under a cocoanut shell on the beach at Puerto Nuevo Point. Thus I was enabled to make some observations on both species under laboratory conditions. Both behaved much in the same manner.

The cocoon of *B. ovigerus* is globular and rather large in proportion to the size of the spider, being fully 2 mm. in diameter, with thin, white walls and a few eggs well visible through the silk. The spider makes no web of any kind, but walks dragging the cocoon behind her and if disturbed runs for shelter. Deprived of the cocoon the spider shows signs of uneasiness and on discovering the cocoon grasps it with her cheliceræ, bends her almost globular abdomen until the spinnerets reach the cocoon from below, releases her hold on it with the cheliceræ and starts off dragging now the cocoon behind her. For two days she behaved this way. On the third day I was surprised to find her sitting on the bottom of the jar while the cocoon was hanging close by, suspended by a few threads in a small web made of loose threads and much of the type common in small Linyphiids. The explanation of the change in behavior was furnished the same day when minute spiderlings emerged from the cocoon. Toward the end, then, of her maternal duties the original instinct common to all Linyphiids asserted itself, showing that the species still retains some of the family habits and that the new habit did not develop to the point characteristic for Lycosids, where the spiderlings are carried by the mother on her back until they have moulted and are large enough to shift for themselves.

Lithyphantes oöphorus has an elongated abdomen vividly marked with black and white, the colors forming a pattern of a type more or less common for the genus. The cocoon is globular,

still more out of proportion with the size of the spider, being 1.2 mm. in diameter, with thin, white walls barely covering the dozen eggs. The cocoon was firmly attached to the spinnerets and the spider would not release it when disturbed. On the second day, however, she was found sitting on the bottom of the jar while the cocoon was suspended by a few losely woven threads. On examination, it was found that two of the eggs developed into spiderlings, while the remainder were parasitized by an insect. Since the spiderlings emerged from the eggs there is nothing singular in the fact that the mother abandoned the cocoon.

Here, then, we have proof positive that one of the most stable instincts in spiders may be modified and what is still more interesting may be modified in the same sense in two different families, presenting a clear case of adaptive parallelism. It is true that the two families are closely related. In various instances species placed in the family Linyphiidæ were later transferred to the family Theridiidæ, when the presence of the "comb" on the fourth tarsi was ascertained. In the great majority of species the distinction between the two families is quite pronounced and certainly there is no close relationship between the genera *Bathyphantes* and *Lithyphantes*.

It is more difficult to decide which of the two methods of taking care of the cocoon should be considered the more primitive one. At first thought it would seem as if carrying the cocoon attached to the spinnerets were simpler than hanging it up in a web. The spinnerets and the cheliceræ are the organs employed in the making of the cocoon. The former produce and weave the silk, the latter are used in clipping the sheet and in joining the seam. We know spiders which carry the cocoon in their cheliceræ, as for example, *Spermophora* and *Scytodes*. The families to which these two genera belong have been often considered to be primitive. It seems to me however, that the family Pholcidæ to which *Spermophora* belongs, shows all signs of specialization, especially in the structure of the cephalothorax and legs. At any rate, we know as yet no truly primitive spiders which would carry their cocoon attached to the spinnerets, and the Lycosidæ which generally possess this habit, are not in any

sense primitive. For this reason it cannot be possibly maintained that *B. ovigerus* and *L. oöphorus* have simply dropped the habit characteristic of their respective families and reverted to a more generalized and fundamental habit original with all spiders. The conclusion is inevitable that in the course of evolution, before any morphologically considerable changes have occurred, our two species of spiders have modified their habit.